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O'Brien and Gere Engineers, Inc., Syracuse, NY

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EVALUATION OF URBAN RUNOFF AND COMBINED SEWER OVERFLOW MUTAGENICITY

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16. ABSTRACT

The introduction of potential mutagens to the human environment may serve to increase the rate of contact with substances that contribute to cancer incidence in the general population. The primary purpose of this study was to evaluate combined sewer overflows and urban runoff for the presence of chemical mutagens. The Ames Salmonella/microsome mutagenicity test was employed as a general biological effects test for the qualitative detection of mutagens in the sanitary environment, including rain, urban runoff, sanitary wastewater, combined sewer overflows, sewage treatment plant effluent, and receiving waters. The Ames test is a relatively sensitive and simple bacterial test for detecting chemical mutagens. Its advantages over long-term animal tests are speed, ease, and relative low cost. The test employs previously mutated Salmonella typhimurium LT2 bacterial strains which have a tendency to undergo a subsequent mutation back to their natural state when exposed to mutagenic compounds.

Nineteen samples elicited a detectable response to one or more of the five Salmonella typhimurium test strains, with or without metabolic activation. Nine of these samples (47%) were of urban runoff in the project area, metropolitan Syracuse (Onondaga County) N.Y., including 17 of 30 detectable responses (57%). Five of the samples (26%) were from combined sewer overflows (7 of 30 detectable responses, or 23%).

The results indicated that substances present in urban runoff which produce a detectable response in the Ames test may be diluted or inactivated in combination with sanitary sewage to form combined sewage, since fewer responses were detected in combined sewer overflow than in urban runoff.

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FOREWORD

The U.S. Environmental Protection Agency was created because of increasing public and governmental concern about the dangers of pollution to the health and welfare of the American people. Noxious air, foul water, and spoiled land are tragic testimony to the deterioration of our natural environment. The complexity of that environment and the interplay between its components require a concentrated and integrated attack on the problem.

Research and development is that necessary first step in problem solution and it involves defining the problem, measuring its impact, and searching for solutions. The Municipal Environmental Research Laboratory develops new and improved technology and systems for the prevention, treatment, and management of wastewater and solid and hazardous waste pollutant discharges from municipal and community sources, for the preservation and treatment of public drinking water supplies and to minimize the adverse economic, social, health, and aesthetic effects of pollution. This publication is one of the products of that research; a most vital communications link between the researcher and the user community.

Studies of the impact of urban runoff and combined sewer overflows on the environment have been limited to water quality and short term public health (microbial pathogen) issues. The potential for long term biological impacts from these sources has gone largely without notice nor investigation. This report presents the results of a screening study into this question.

Francis T. Mayo
Director
Municipal Environmental
Research Laboratory

ABSTRACT

The introduction of potential mutagens to the human environment may serve to increase the rate of contact with substances that contribute to cancer incidence in the general population. The primary purpose of this study was to evaluate combined sewer overflows and urban runoff for the presence of chemical mutagens. The Ames Salmonella/microsome mutagenicity test was employed as a general biological effects test for the qualitative detection of mutagens in the sanitary environment, including rain, urban runoff, sanitary wastewater, combined sewer overflows, sewage treatment plant effluent, and receiving waters. The Ames test is a relatively sensitive and simple bacterial test for detecting chemical mutagens. Its advantages over long-term animal tests are speed, ease, and relative low cost. The test employs previously mutated Salmonella typhimurium LT2 bacterial strains which have a tendency to undergo a subsequent mutation back to their natural state when exposed to mutagenic compounds.

Nineteen samples elicited a detectable response to one or more of the five Salmonella typhimurium test strains, with or without metabolic activation. Nine of these samples (47%) were of urban runoff in the project area, metropolitan Syracuse (Onondaga County), N.Y., including 17 of 30 detectable responses (57%). Five of the samples (26%) were from combined sewer overflows (7 of 30 detectable responses, or 23%).

The results indicated that substances present in urban runoff which produce a detectable response in the Ames test may be diluted or inactivated in combination with sanitary sewage to form combined sewage, since fewer responses were detected in CSO than in urban runoff.

Due to budget constraints, it was not possible to conduct an in depth investigation. A complete study should include duplicate analyses and extraction of samples at pH 3 and 12, as well as ambient pH.

This report is in fulfillment of the U.S. Environmental Protection Agency (EPA) Grant R806640. The period of study covered by this report is from April 1980 to January 1982.

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ABBREVIATIONS

Ames Test - Ames *Salmonella*/microsome mutagencity test
BOD - five day biochemical oxygen demand
CD - cadmium
CR - chromium
CSO - combined sewer overflow
CU - copper
°C - degrees Celsius
DCM - dichloromethane (methylene chloride)
DMSO - dimethyl sulfoxide (methyl sulfoxide)
DNA - deoxyribonucleic acid
EPA - U.S. Environmental Protection Agency
G-6-P - glucose-6-phosphate
km - kilometer
l - liter
MAR - mutagenic activity ratio
Metro - Metropolitan Syracuse Treatment Plant
mg/l - milligrams per liter
ml - milliliter
mM - millimolar
MMS - methyl methanesulfonate
MNNG - N-methyl-N'-nitro-N-nitrosoguanidine
N - Normal
NADP - nicotinamide adenine dinucleotide phosphate
O&G - oil and grease
ONA - Oxoid Nutrient Broth No. 2 plus 1.5% agar
ONB - Oxoid Nutrient Broth No. 2
PB - lead
TOC - total organic carbon
TSS - total suspended solids
µ - micron
µl - microliter

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Assistance to the study was provided by Dr. H. Ernest Hemphill, Department of Biology, Syracuse University, Syracuse, NY.

SECTION 1

INTRODUCTION

It has only been since 1964 that urban runoff and combined sewer overflows (CSO) have been identified as contributors of pollution to receiving streams. It was not until nearly ten years later that urban runoff and CSO were implicated as major sources of particulates, nutrients and microbial loading to urban waterways. In recent years, almost any potential pollutant to be found in the urban environment can be traced to receiving streams via this transport pathway (37).

In order to decrease surface water pollution from these sources, the U.S. Environmental Protection Agency (EPA) has engaged studies and provided funds for the design and construction of prototype CSO treatment facilities. These facilities are primarily designed to utilize some form of solids removal followed by disinfection.

However, past studies which have served to characterize urban runoff and CSO, and identify constituent pollutants, have not conclusively dealt with the most recent concern to arise in pollution control, that of the fate and effect of organic chemicals in these systems. Although potentially toxic, mutagenic, carcinogenic or teratogenic chemicals are readily identifiable in the urban environment, there has been little attempt to determine their presence and concentrations in urban runoff and CSO. There are several reasons for this omission. Such studies would require the analysis of long lists of compounds, as in priority pollutant analysis, and these lists are constantly expanding in length. There are technological difficulties in the analysis of such compounds. The detection limits of available instrumentation may not be sufficiently low to detect minute concentrations of contaminants at levels which may still pose a potential health hazard. The cost of analyzing a single sample under these conditions is high; the analytical cost of sufficient samples for a long list of compounds to provide statistically significant amounts of data is staggering. Further, the results of such determinations still would not provide any biohazard risk index - any measure of the biological effects of these compounds when in their environmental medium, particularly on the human system, and especially any synergistic or multiplicative effects of such substances.

At this time, most treatment systems for CSO are still in the initial planning or evaluation phases. While there has been general agreement that treatment should consist of solids removal and disinfection, there has been little evaluation of the need for additional levels of treatment. Due to the highly variable nature and occurrence of urban runoff and CSO, it is doubtful if the requirement for advanced treatment can be ascertained solely by characterization of a list of specific chemicals. Instead, general information describing the potential biohazard risk of these systems would be a valuable criterion for the determination of the need for advanced treatment.

It is upon this premise that this study has been based, that urban runoff may substantially contribute substances of potential risk to the human environment and that a preliminary analytical survey utilizing the Ames Salmonella/microsome mutagenicity test might serve to outline the presence and scope of that potential.

To this end, samples were taken of environmental contributors to the CSO system. These included rain, urban runoff, dry weather sanitary flows, and CSO, as well as influent and effluent from the Metropolitan Syracuse Treatment Plant (Metro), and samples from Onondaga Lake, the ultimate receptor of the overflows. Samples were tested by the Ames test and subjected to analysis for other chemical parameters, including total suspended solids (TSS), total organic carbon (TOC), five day biochemical oxygen demand (BOD), oil and grease (O&G), and the heavy metals cadmium (CD), chromium (CR), lead (PB), and copper (CU). The results of the Ames test were compared to the baseline chemical analyses to determine the presence of any correlation which might assist in the identification of the source of mutagenic activity.

Since inorganics, especially metals, are removed in the sample extraction procedure, the primary purpose of heavy metal analysis is for the characterization of the samples, and evaluation as to whether the concentrations were typical or atypical of the sample type.

This study was completed in two phases. Phase I involved the collection and analysis of 74 samples during 1980. Based on the results of those analyses, modifications were made to some of the procedures. Phase II involved the collection and analysis of an additional eleven samples in 1981 from those drainage areas which elicited detectable responses in the 1980 Phase I effort.

The procedures and results of this study will be described in later sections of this report.

SECTION 2

CONCLUSIONS

1. Nineteen samples may be interpreted to have induced a detectable mutagenic response from one or more of the test strains, with or without metabolic activation. Nine of these samples were of urban runoff. Some samples required a two hundred-fold concentration for detection of this activity. Of a total of 30 detectable responses, 17 (57%) were found in urban runoff, while 7 (23%) were found in combined sewer overflows.

2. The samples eliciting a detectable response did not appear correlated to levels of any of the chemical parameters measured, nor to any particular CSO site. The experimental design appeared too limited in terms of number of samples and the chemical parameters measured to obtain correlations between detectable mutagenic activity and chemical characteristics of samples or sample types (CSO, urban runoff, etc.).

3. Substances present in urban runoff which produce a detectable response in the Ames test may be diluted or inactivated in combination with sanitary sewage when it forms combined sewage, since fewer responses were detected in CSO than in urban runoff.

4. This study is indicative of the need for a more comprehensive survey of a similar nature. However, problems encountered in analytical methodology, sample toxicity, and the inherent chemical variability of CSO and urban runoff pose difficulties in preparing definitive conclusions from this preliminary survey which can assist in policy decisions.

5. Use of the spot test as a preliminary step in the analysis of polluted aquatic samples by the Ames Salmonella/microsome mutagenicity test may be precluded by sample toxicity at ambient concentrations.

6. Dichloromethane (methylene chloride) may serve as both a disinfectant and extractant. However, should extraction and/or concentration not be required in other aquatic environmental samples, a more suitable disinfection procedure may be necessary.

SECTION 3

RECOMMENDATIONS

1. An evaluation should be performed to determine an optimum method of test sample disinfection for those samples with high levels of particulates, oils and microbial organisms, such as those analyzed by the Ames test in this study.

2. The extraction procedure recommended for this project should be evaluated as to its sensitivity in recovering known mutagens. This would establish a detection limit threshold for the assay under these analytical conditions and assist in risk evaluations based on the review of analytical data.

3. In the analysis of environmental samples in which the constituents may be complex and ill-defined, all five available test strains should be used; strains without the R factor (TA1535, TA1537, TA1538) may be more sensitive to weakly mutagenic components of the samples than either TA98 or TA100.

4. An expanded evaluation of the mutagenic activity of combined sewer overflows, urban runoff, sanitary sewage and treatment plant influent and effluent should be performed. Some additional emphasis should be placed on urban runoff samples. It may be advantageous to analyze the samples concurrently for priority pollutants to determine any possible correlation.

5. Mutagenic activity of combined sewer overflows and urban runoff should be evaluated on a broader geographic base, and under wider variation in land use characteristics.

6. An evaluation of precipitation should be performed to determine the transport and contribution of toxic, and possibly mutagenic, pollutants by this source. Concurrent analysis of samples for priority pollutants and mutagenic activity is recommended.

7. The U.S. Environmental Protection Agency should consider the Ames test and/or other indicators of biohazard risk in future regulatory strategy for the control of emissions to the environment and the establishment of standards, goals and criteria.

SECTION 4

BACKGROUND

HISTORY OF COMBINED SEWERS

Beginning around the middle of the 19th Century, sewers were constructed in urban areas of the U.S., originally to conduct stormwater runoff to the nearest watercourse. Sanitary wastes were disposed of separately at individual residences. Later, as population increased, health problems developed as a result of waste disposal at so many individual locations.

A convenient method was needed to eliminate sanitary wastes from the urban environment. Existing storm sewers were available and were frequently adapted to transport sanitary wastes away from the community. Sewers which carried both stormwater and sanitary wastes were designated combined sewers. Nuisance conditions in the watercourses resulted, which led to the construction of interceptor sewers, to "intercept" dry-weather sanitary flows upstream from receiving watercourses. However, these intercepting sewers were not designed with sufficient capacity to carry all sanitary wastes and stormwater during wet weather periods. In general, the interceptors were designed to carry only 2 to 3 times the average dry weather flow from tributary drainage areas. Thus, when storms occurred, runoff reaching the collection system resulted in flows in the trunk sewers in excess of the carrying capacity of the interceptors, creating the potential for surcharging of sewers, flooding of streets and private basements, and resulting in public health dangers. In order to relieve the trunk sewers of excess flow, overflow pipes were installed at frequent intervals along the interceptors which discharged a combination of excess stormwater and sanitary wastes (Figure 1). In hydraulically limited circumstances, such as underdesigned pipe capacities or blocked sewers, undiluted sanitary sewage was discharged during dry weather periods as well (24).

As urban centers continued to grow and discharges of CSO wastewaters to receiving waters increased, the natural assimilation capacity of many streams was exceeded and serious water quality problems resulted. Since 1950, emphasis in newly sewered areas has been placed on construction of separate sanitary and storm sewers. By building separate sewers, excessive flows to the waste treatment plant during wet weather periods are avoided and the entire sanitary flow is treated without direct discharge to and subsequent degradation of the receiving water. Stormwater runoff in separately sewered areas is conducted by the storm sewers directly to receiving waters. This does not alleviate pollution as a result of the stormwater contamination during urban runoff in these cases.

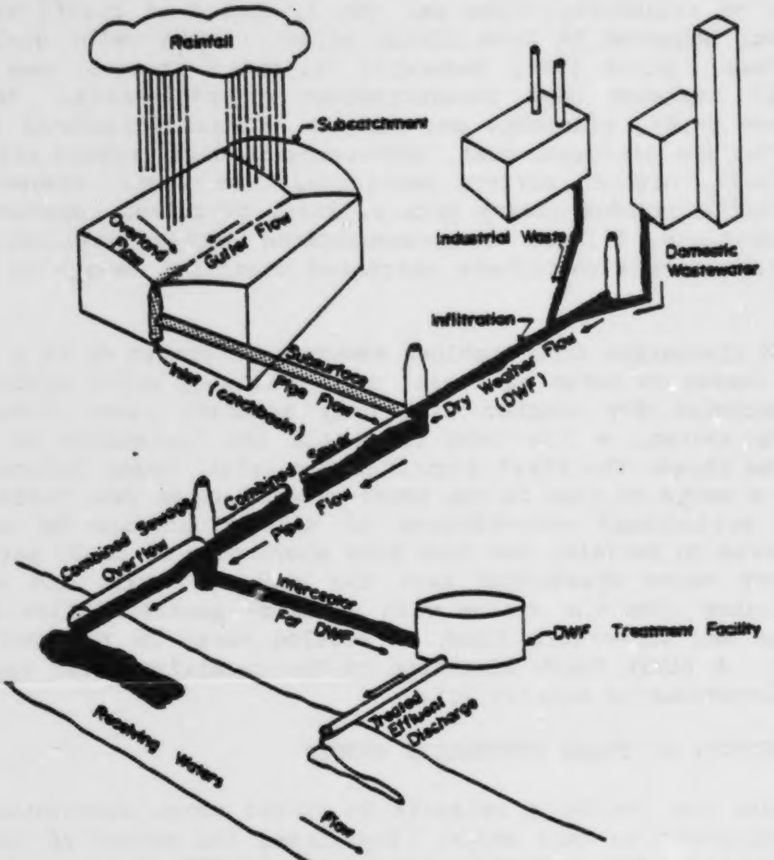

Rainfall
Subcatchment
Overland Flow
Gutter Flow
Industrial Waste
Domestic Wastewater
Infiltration
Subsurface Pipe Flow
Inlet (catchbasin)
Dry Weather Flow (DWF)
Combined Sewer
Pipe Flow
Combined Sewage Overflow
Interceptor For DWF
DWF Treatment Facility
Treated Effluent Discharge
Receiving Waters
Flow

Figure 1. Typical Combined Sewer

Prior to 1964, when the U.S. Public Health Service published its study "Pollutional Effects of Storm Waters and Overflows from Combined Sewer Systems: A Preliminary Appraisal", surface runoff from rainfall events was considered to be relatively clean and the discharge of runoff water from a storm sewer was believed to have little effect on the water quality of the receiving stream. Since 1964, federally supported studies have shown that surface runoff contains high concentrations of pollutants. Rain removes pollutants from yards, sidewalks and streets. These pollutants may include oxides of sulfur and nitrogen, dust, hydrocarbons, dirt, common street litter, deicing chemicals, organic matter, pesticides from lawns, eroded nonorganic materials, traffic residue (heavy metals, lead, petroleum compounds, rubber) and animal droppings. Illegal cross-connections between overloaded sanitary sewers and storm sewers contribute untreated municipal sewage to the stormwater flows.

Untreated discharges from combined sewers have proven to be a significant source of pollution in terms of impact upon receiving water quality. During periods of extended dry weather with only sanitary waste flowing in the combined sewer system, solids tend to settle and accumulate on the bottom (invert) of the pipes. The first significant rainfall event following the dry period causes a surge of flow in the sewer which flushes this residue from the system. The pollutional contribution of this flush can be significant. Studies conducted in Buffalo, New York have shown that 20 to 30 percent of the annual sanitary waste discharged into the combined sewer are settled and eventually flushed from the system with a storm- generated flow surge (33). The flow surge and associated flush of settled waste is referred to as the "first flush". A first flush discharge to the receiving water can produce a shock load deleterious to aquatic life.

QUALITATIVE ASPECTS OF URBAN STORMWATER RUNOFF

Little data was available relative to direct urban stormwater runoff in Onondaga County prior to this study. Therefore, the extent of the contribution of pollutants in the stormwater runoff to the CSO system cannot be fully evaluated. However, studies conducted in other parts of the United States have indicated that large quantities of relatively contaminated water drains to watercourses with no form of treatment to reduce the pollutant load. Various studies have indicated that total suspended solids and settleable solids concentrations were frequently higher in urban stormwater runoff than in combined sewer overflows. Table 1 presents the pollutant characteristics of stormwater runoff in several cities in the U.S., while Table 2 presents pollutant characteristics of some combined sewer overflows in the U.S. (33). These tables indicate that additional work to reduce direct stormwater runoff may be necessary, after CSO abatement is achieved, to assure that receiving water quality standards are maintained.

TABLE 1. POLLUTANT CONCENTRATIONS IN STORMWATER RUNOFF.

	Average pollutant concentrations, mg/l									
City	TSS	VSS	BOD5	COD	Kjeldahl nitrogen	Total nitrogen	Phos-phorus	Ortho-phosphate	Lead	Fecal coliforms[a]
Atlanta, Georgia	287	---	9	48	0.57	0.82	0.33	---	0.15	6,300
Des Moines, Iowa	419	104	56	---	2.09	3.19	0.56	0.15	---	---
Durham, North Carolina	1,223	122	--	170	0.96	---	0.82	---	0.46	230
Knoxville, Tennessee	440	---	7	98	1.9	2.5	0.63	0.30	0.17	20,300
Oklahoma City, Oklahoma	147	---	22	116	2.08	3.22	1.00	1.00	0.24	40,000
Tulsa, Oklahoma	367	---	12	86	0.85	---	---	0.38	---	420
Santa Clara, California	284	70	20	147	---	5.8	0.23	---	0.75	---
Average (not weighted)	415	88	20	113	1.41	3.11	0.62	0.46	0.35	13,500
Range	147-1,223	70-122	7-56	48-170	0.57-2.09	0.82-5.8	0.33-1.00	0.15-100	0.15-75	230-40,000

[a] Organisms/100 ml.

TABLE 2. POLLUTANT CONCENTRATIONS IN COMBINED SEWER OVERFLOWS.

City	Average pollutant concentrations, mg/l TSS	VSS	BOD5	COD	Kjeldahl nitrogen	Total nitrogen	Phosphorus	Orthophosphate	Lead	Fecal coliforms[a]
Des Moines, Iowa	413	117	64	---	---	4.3	1.86	1.31	---	---
Milwaukee, Wisconsin	321	109	59	264	4.9	6.3	1.23	0.86	---	---
New York City, New York										
Newton Creek	306	182	222	481	---	---	---	---	0.60	---
Spring Creek	347	---	111	358	16.6	---	---	---	---	---
Racine, Wisconsin	551	154	158	---	---	---	2.78	0.92	---	201,000
Rochester, New York	273	---	65	---	2.6	---	---	0.88	0.14	1,140,000
Average (not weighted)	370	140	115	367	3.8	9.1	1.95	1.00	0.37	670,000
Range	273-551	109-182	59-222	264-481	2.6-4.9	4.3-16.6	1.23-2.78	0.86-1.31	0.14-0.60	201,000-140,000

---

[a] Organisms/100 ml.

The limited data presented in Table 1 indicate that pollutant loadings from urban stormwater runoff could result in serious deleterious effects on the surface waters to which they discharge. The impacts may be localized in nature, or could extend for long distances and for long periods of time after the storm event. To date, little information is available to define the extent of the problem, if any, in Onondaga County. The limited data generated as part of this study, which will be presented in later sections of this report, do indicate similar potential water quality impacts to those implied by Table 1. The various studies from which Table 1 were developed indicate that this source of pollution should not be neglected.

POLLUTED PRECIPITATION

Studies in recent years have indicated that precipitation may be the vehicle for significant contamination of surface waters with pollutants from industrial and urban sources. This contamination may originate hundreds of miles from its eventual impact, transported by regional weather patterns. Atmospheric washout of pollutants extends far beyond acidic components which contribute to the depressed pH characteristic of rainfall in the Northeast. The term "acid rain" may well serve as an umbrella for a whole gamut of atmospheric pollutant washout problems caused by contamination of surface waters by particulates, oxidants, and a variety of substances, many probably unidentified in precipitation at this time (Table 3)(13).

In Onondaga County, the washout of atmospheric pollutants has not been investigated so that the extent of their effect is unknown. With extensive industrial development in western New York, coupled with regional weather patterns, the potential for this type of pollution exists although its importance as compared to other quality issues may be slight. However, because of the relatively novel aspect of the entire washout concept, the potential for serious pollutant problems should not be discounted.

AMES TEST

The contribution of pollutional loadings to receiving streams from CSO, stormwater runoff and atmospheric washout is composed of a wide variety of compounds. The impacts to the environment and to public health of these compounds may be as individual species or synergistic. Until recently, the emphasis in environmental surveillance was on the quantification of specific contaminants with little or no concern given to their actual impact. It is now generally accepted that environmental factors may alter a substance sufficiently to affect its identification; therefore, it would not be unreasonable to expect the impact on biological systems also to be altered. Therefore, the use of biological effects tests which correlate to in situ impacts are now being used in a wide array of environmental applications.

TABLE 3. PERCENTAGES OF STREAM LOADS OF CHEMICALS RESULTING FROM DIRECT ATMOSPHERIC WASHOUT (1).

Substance	Northeastern U.S. 1963-68
chloride	30-60
calcium	20
sodium	20-30
magnesium	20-50
potassium	70-80
sulfate	90
nitrogen	120-180

The Ames Salmonella/mammalian-microsome mutagenicity test was developed by Dr. Bruce N. Ames and co-workers of the University of California at Berkeley in the early 1970's (2). Originally developed during studies of how bacterial genes are switched on and off in response to the presence of the amino acid histidine in the growth medium and of the effect of mutations which perturbed this control mechanism, it has become a very sensitive and simple bacterial test for detecting chemical mutagens. Its advantages over other tests are speed, ease and relative low cost. Thus, the bacterial mutagenesis test provides a mechanism whereby large numbers of chemicals can be screened for mutagenic activity in a relatively short time (5).

The Ames test relies on the tendency of some previously mutated bacterial cells to undergo a subsequent mutation back to their natural state (revert) when exposed to certain mutagenic chemicals. The compounds are tested on petri plates with Salmonella typhimurium bacteria, LT2 strain. Five primary tester strains have been developed, after a screening process involving hundreds of mutants, which may be reverted from a histidine amino acid requirement (histidine auxotrophy) to nutritional normality (prototrophy) by a variety of mutagens, with sensitivity and specificity (41).

Normally, Gram negative bacteria have a rather impermeable envelope that reduces the penetration of many chemicals. Ames introduced a mutation which gives rise to envelope defects, resulting in a greater sensitivity of the organism to DNA-damaging agents, and also introduced a plasmid (foreign genetic element) which makes DNA replication more error prone - the test chemical damages the bacterial DNA which can no longer be repaired (41). This results in the creation of a strain in which a few molecules of a mutagen may create a DNA lesion and each lesion may cause a mutation. Some of these mutations will be such that the internal ability to supply histidine is restored. The number of these chemically induced revertants is compared to the background (spontaneous) reversion rate to determine the mutagenic strength of the test compound (5).

Several workers discovered that many substances must be converted by enzymes in liver or other tissues to an active (electrophilic) form which is the true mutagen (17,21,23). Ames added homogenates of rat liver to the petri plates, incorporating an important *in vivo* aspect of mammalian metabolism into the *in vitro* test (5,12). In fact, although the sensitivity may be affected, it appears that any mammalian liver may be used to prepare the microsomal fraction, known as the S-9 fraction, as well as other tissue fractions (5,12,16,42,43).

About 85% of the known carcinogens subjected to the Ames test have been detected as mutagens. Less than 10% of non-carcinogens have been found to be mutagenic in the test (28,38,40,54,55). This affirms the desirability of using this rapid, economical test system as a screening technique to identify those potentially dangerous substances among the thousands of chemicals and mixtures found in the human environment.

The Ames test can be used to evaluate the mutagenic potential of mixed or undefined solutions and pure compounds. It has been used to screen drinking water (10,22), municipal (56) and industrial (18) wastewaters, industrial products and a wide variety of other substances, such as:

- foods and food extracts
  - milk (51)
  - saccharin (6)
  - caffein (8)
  - cooked beef (11)
- textile dyes (20)
- fly ash (32)
- photocopies (35)
- cosmetics (45)
- crankcase oils (52)
- pesticides and herbicides (60)
- animal feed additives (49)
- air emissions and pollutants (64)
- plant extracts (27)
- algal by-products (34)
- cigarette smoke condensates (30)
- fungal toxins (68)
- surface waters (53)
- recycled waters (25)

PROJECT AREA LOCATION

Onondaga County is located near the geographical center of New York State (Figure 2). The county is about 34 miles (55 km) from north to south, 30 miles (48 km) wide in an east-west direction, with a land area of 793.5 square miles (2063 square kilometers), (9). With a population of 474,703 (1975 est.) (9), the county ranks as the tenth most populous in the state.

Syracuse, the county seat, is centrally located (Figure 3) and has a population (1975 est.) of 183,334 (9) living in a land area of approximately 25.0 square miles (65 square kilometers).

PROJECT BACKGROUND

In the past decade, Onondaga County, New York, has administered a series of studies and construction projects whose objective has been to improve the level of water quality in Onondaga Lake, its tributaries and downstream surface waters. The major sources of pollution were identified as industrial waste discharge, insufficiently treated municipal wastewater, and combined sewer overflows. An industrial waste program was initiated in the county in 1973 to reduce liquid waste sources, quantities, composition and disposition. Municipal wastewater treatment has been upgraded in the Syracuse Metropolitan area, particularly with the completion of the recent expansion of the Metropolitan Syracuse Sewage Treatment Plant. Finally, in 1979, preliminary design plans were prepared and submitted for structural and non-structural improvements to the Metropolitan Syracuse Sewerage System that would decrease the quantity and upgrade the quality of CSO discharges. Presently, the program is in its preliminary phase involving the application of best management practices to the system. In addition to the generation of baseline data in regard to the Ames test, this study may be useful in the determination of the necessity of advanced treatment of CSO discharges to prevent the introduction of mutagenic substances to the aquatic environment.

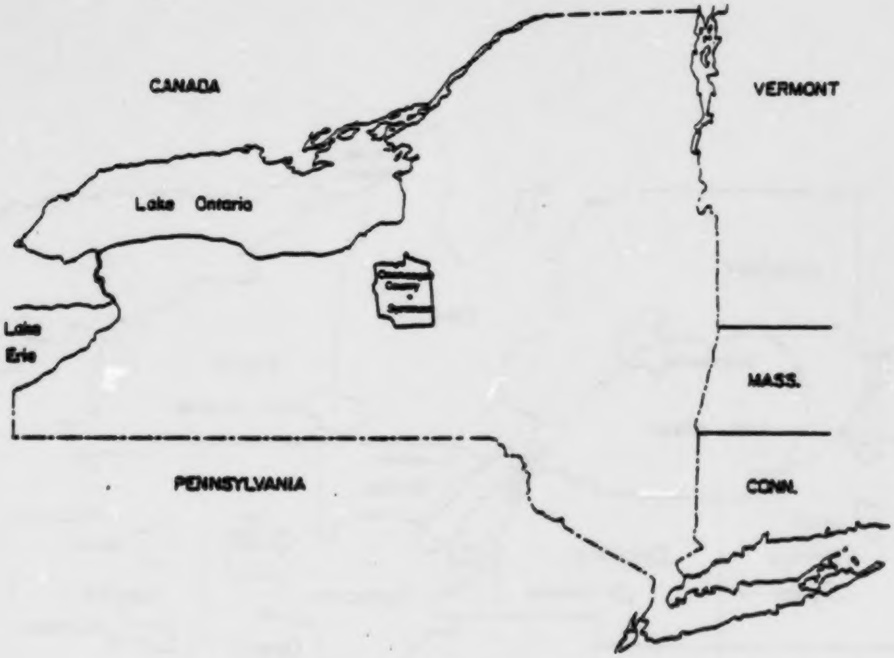

CANADA
VERMONT
Lake Ontario
Lake Erie
MASS.
CONN.
PENNSYLVANIA

Figure 2. Location of Onondaga County in New York State.

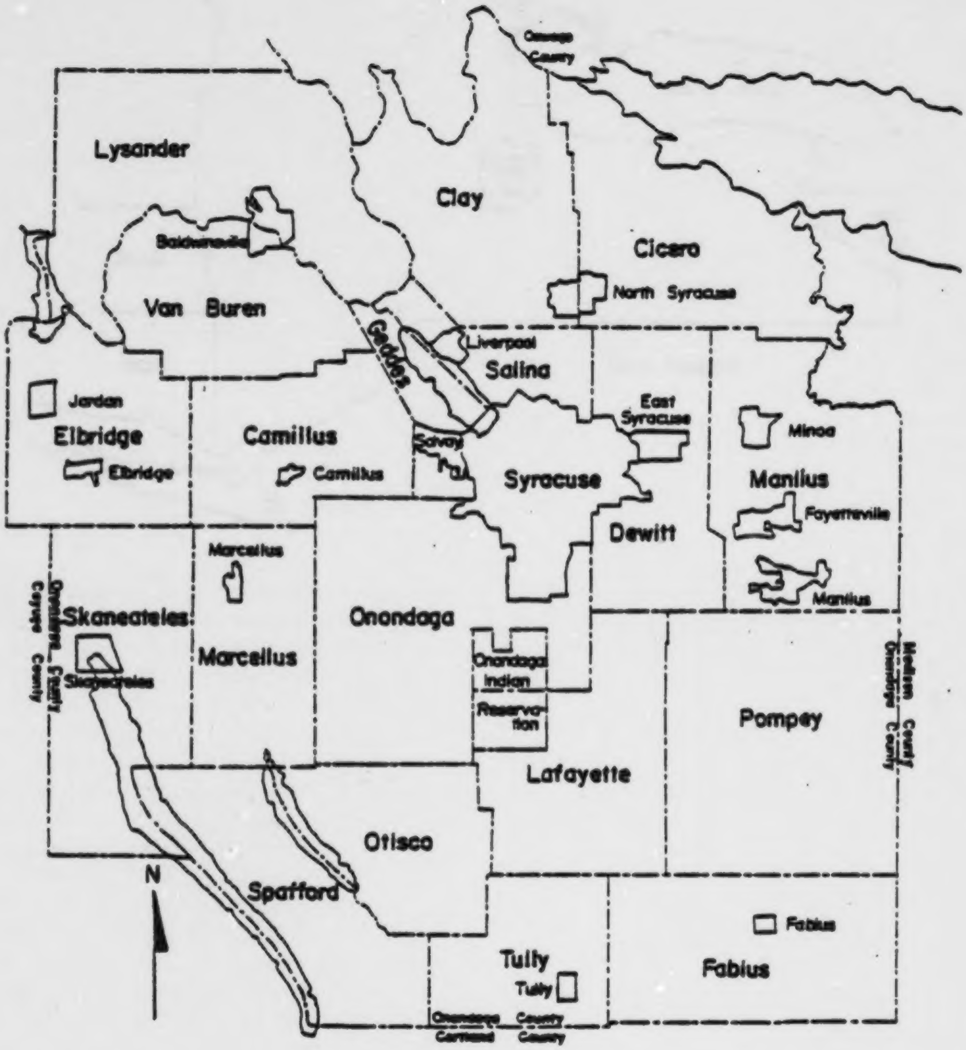

Oswego County
Lysander
Clay
Cicero
Baldwinsville
North Syracuse
Van Buren
Geddes
Liverpool
Salina
East Syracuse
Jordan
Elbridge
Camillus
Solvay
Syracuse
Minoa
Manlius
Elbridge
Camillus
Fayetteville
Dewitt
Marcellus
Manlius
Onondaga County
Cayuga County
Skaneateles
Onondaga
Marcellus
Onondaga Indian Reservation
Skaneateles
Madison County
Onondaga County
Pompey
Lafayette
Otisco
N
Spafford
Fabius
Tully
Fabius
Tully
Onondaga County
Cortland County

Figure 3. Onondaga County, New York.

SECTION 5

MATERIALS

AMES TEST

General

Procedures employed in the analytical phase of the project were derived from three sources:

a) a form instruction sheet ("Supplement to the Methods Paper", June 1979) supplied with the test strains from the laboratory of Dr. Bruce Ames (3);

b) an "Ames Test Information Package" prepared by the EPA (57); and

c) a primary methodology journal article (5).

The analytical procedures as outlined in this section were forwarded to the Project Officer for review prior to use in the project. Recommendations made on the basis of this review were incorporated into the test methodology and are referenced as applicable.

Test Strains

For analyses performed under Phase I, the wild-type *Salmonella typhimurium* LT2 and five histidine dependent test strains were obtained from Dr. Bruce N. Ames, Department of Biochemistry, University of California, Berkeley: TA98, TA100, TA1535, TA1537, TA1538. For Phase II, copies of the five bacterial test strains were obtained from the USEPA Region II Environmental Services Division, Edison, NJ. Strains were grown on Oxoid Nutrient Broth No. 2 plates (Oxoid Ltd., Basingstoke, England) with 1.5% agar (BBL, Cockeysville, MD) (hereafter known as ONA) (3). Colonies were picked for overnight cultures as required, grown in Oxoid Nutrient Broth No. 2 (hereafter known as ONB). Petri plates used were Falcon Muta-Assay Dishes (Falcon Labware, Oxnard, CA) (Cat. No. 1029), sterilized without ethylene oxide, which may cause a mutagenic response in the test (3).

Solutions

The minimal salts medium was prepared as by Vogel and Bonner (67) except at a strength of 10X rather than 50X (Table 4); color changes noted in the 50X medium when autoclaved were not evident in the 10X preparation. When diluted to normal strength, agar was added at a final concentration of

1.5%/liter. A supplemental glucose solution was autoclaved separately such that the final medium would contain 5% glucose (Sigma Chemical Co., St. Louis, MO.; cat. no. G-5000).

Top agar contained 0.5% NaCl (Mallinckrodt) and 0.6% agar, autoclaved and stored in 100 ml portions in milk dilution bottles (5,57). The top agar was melted in a water bath as needed prior to use.

The 0.5 mM (millimolar) L-histidine and 0.5 mM biotin (both Sigma) solutions were autoclaved and stored refrigerated for later use (5,57).

The S-9 reaction mixture was prepared as indicated (Table 5)(5,57). The salts solution and phosphate buffer were prepared and autoclaved for later use. NADP and G-6-P were prepared fresh; both solutions were filter sterilized (0.45u pore size membrane filter; Millipore Corp., Bedford, MA) and prepared using preautoclaved distilled, deionized water. Any excess solution was refrigerated and used within one week. Commercial S-9 extract was stored frozen and hand-thawed prior to use. The reaction mixture was stored on ice prior to and during use.

Standards and spikes were prepared from either benzo(a)pyrene (3,4-benzpyrene) (hereafter known as BaP) (Sigma, cat. no. B-3500), methyl methanesulfonate (hereafter known as MMS) (Eastman Kodak Co., Rochester, NY), or N-methyl-N'-nitro-N-nitrosoguanidine (hereafter known as MNNG) (Aldrich Chemical Co., Inc., Milwaukee, WI).

Other chemicals required included ampicillin (Sigma, cat. no. A-6140); dimethyl sulfoxide (hereafter known as DMSO) (Mallinckrodt, spectrophotometric grade); dichloromethane (hereafter known as DCM) acetone and hexane (all Mallinckrodt, nanograde); and crystal violet (0.01% solution) (Matheson Coleman & Bell, Cincinnati, OH).

Glassware

All pipettes, beakers, flasks, test tubes, dilution bottles, sample containers and sample storage vials were glass. No plasticware was used in contact with sample aliquots to be used for the Ames test.

Glassware utilized in the extraction of raw samples was soap and water washed, Chromerge (chromic acid-sulfuric acid solution) soaked overnight, water rinsed and finally, rinsed three times in acetone followed by three hexane rinses.

Bottles used for the collection and storage of samples were one gallon (3.8 l) amber glass obtained from Empire State Bottle Co. of Syracuse, Inc. (Syracuse, NY). These bottles and the sample extract storage vials required their caps to be foil lined prior to use. Both were DCM rinsed prior to use; the vials were also autoclaved.

TABLE 4. VOGEL-BONNER MINIMAL SALTS BASE MEDIUM (67).

Compound[a]	Amount (g)
$MgSO_4 \cdot 7H_2O$	2
citric acid	20
K_2HPO_4 anhydrous	100
$NaNH_4HPO_4 \cdot 4H_2O$	35
distilled, deionized water to 1 l.	

[a] All chemicals were Mallinckrodt, Inc. (Paris, KY) analytical reagent grade.

TABLE 5. S-9 REACTION MIXTURE.

Compound	Amount (ml)	Source
Aroclor 1254-Induced Rat Liver S-9	1.0	Litton Bionetics, Kensington, MD (Cat. No. 8360-01)
0.1M NADP[a]	0.4	Sigma (cat. No. N-0505)
1.0M G-6-P[b]	0.05	Sigma (cat. No. G-7879)
0.4M $MgCl_2$ + 1.65M KCl (salts)	0.2	Both Mallinckrodt
phosphate buffer[c]	5.0	Both Mallinckrodt
sterile H_2O	3.35	distilled, deionized
Total	10.	

[a] beta-nicotinamide adenine dinucleotide phosphate, monosodium salt

[b] D-glucose-6-phosphate, monosodium salt

[c] 0.4M $NaH_2PO_4 \cdot H_2O$ + 0.4M $Na_2HPO_4 \cdot 7H_2O$, pH 7.4

CHEMICAL ANALYSES

All chemicals used for analyses other than the Ames test were reagent grade, unless otherwise specified in the procedures.

SECTION 6

PROCEDURES

PLAN OF STUDY

A general plan of study (Detailed Project Work Plan: Urban Runoff and Combined Sewer Overflow Mutagenicity, May, 1980) was prepared for this project in conjunction with the Project Officer. The plan of study set forth project objectives, outlined procedural protocols, and delineated problems which might be encountered. Due to the known variability in the chemical composition of CSO and urban runoff, the inherent difficulties in working with a biological system, and the aspects involved in performing a noval but limited survey, it was anticipated that analytical and other general problems would be encountered which would be difficult to overcome within the original framework of the plan of study. For this reason, the plan of study was prepared to be flexible, with the success of each phase governing later options. As anticipated, several modifications to the plan of study were made to accommodate these experimental difficulties. This report reflects those modifications which alter the letter, but not the spirit of the original project. The major limiting factors were the prolonged and severe drought in Onondaga County in the summer of 1980 and the general absence of a high level of mutagenic activity, as compared to studies in other aquatic environmental systems (10,18,22,25,53,56).

As a result of the 1980 study, additional sample collection and analysis were performed in 1981 for verification of initial conclusions based on 1980 data.

SAMPLE COLLECTION

Provisions were made in the original plan of study for the collection of seven types of samples in Onondaga County (Syracuse):

a. combined sewer overflows,
b. dry weather sanitary flow,
c. urban runoff,
d. influent and effluent to Metro,
e. Onondaga Lake, and
f. rain

The project plan of study required the selection and collection of one CSO sample during four wet weather events from each of six CSO discharges. The objective was to select six drainage areas to be examined, each with divergent characteristics of size, land use, population density and/or percent imperviousness. Selection was made based upon previous studies performed in the Syracuse area to characterize CSO sites (48).

The physical characteristics of the six CSO sites which were selected are presented in Table 6 and are summarized as follows:

(a) site 005 - a small drainage area with a high percentage of impervious surface (86%) and predominantly commercial land use; however, little historical chemical data was available from previous studies to characterize this area;

(b) site 019 - a large drainage area with mixed land use;

(c) site 027 - the only drainage area which includes a significant amount of industrial land use;

(d) site 037 - a drainage area with a high percentage of impervious surface and mixed land use;

(e) site 043 - a large, high density residential area; and

(f) site 046 - the only drainage area which is primarily open space.

In addition, two alternate sites were chosen in the event that difficulties arose at any time in the collection of samples from the primary sites. These alternates were:

(a) site 004 - This drainage area is a large basin characterized by mixed residential/commercial land use; and

(b) site 074 - a drainage area with predominantly commercial/industrial land use.

The locations of the project CSO sites may be found on Figure 4.

The sample collection sites were chosen using three selection criteria:

(a) uniqueness of drainage area characteristics - drainage areas with percent imperviousness, population density, size and land use mix representative of several others in the city were evaluated for selection. In addition, drainage areas which were unique in the city (i.e. predominantly open space, such as site 046) were selected.

(b) ease and safety of sample collection - where several sites of similar physical characteristics could have been selected, ease of sample collection was considered for the preservation of sample integrity. In addition, both receiving streams traverse highly trafficked urban areas where sample collection would be performed through manholes in streets. To ensure the safety of personnel, accessibility during high traffic periods was considered.

TABLE 6. CHARACTERIZATION OF CSO SITE DRAINAGE AREAS IN SYRACUSE, NY.

Overflow Number	Collector System[a]	Runoff Area[b] (acres)	Percent Impervious	Average Land Slope	Sanitary Area[b] (Acres)	Land Use[c]	Population (Density/Acre)
005	HBIS	13.6	86.0	.005	13.6	3	-
019	MIS	387.2	40.0	.005	343.0	1,3	12.8
027	MIS	158.2	48.2	.010	580.8	1,2,3,4	44.7
037	MIS	39.7	90.0	.005	94.0	1,2,3	47.4
043	MIS	278.0	45.1	.005	1363.4	1	71.7
046	MIS	25.1	31.8	.023	25.1	1,5	23.1
004	HBIS	253.3	42.0	.070	242.9	1,3	19.9
074	MIS	74.2	40.0	.016	-	3,4	-

[a] collector system: HBIS - Harbor Brook Intercepting Sewer, MIS- Main Intercepting Sewer

[b] runoff areas and sanitary areas need not coincide exactly for the same drainage basin due to partial sewer separation in different sections of the city.

[c] land use: 1- single family residential
2- multi-family residential
3- commercial
4- industrial
5- undeveloped or park lands

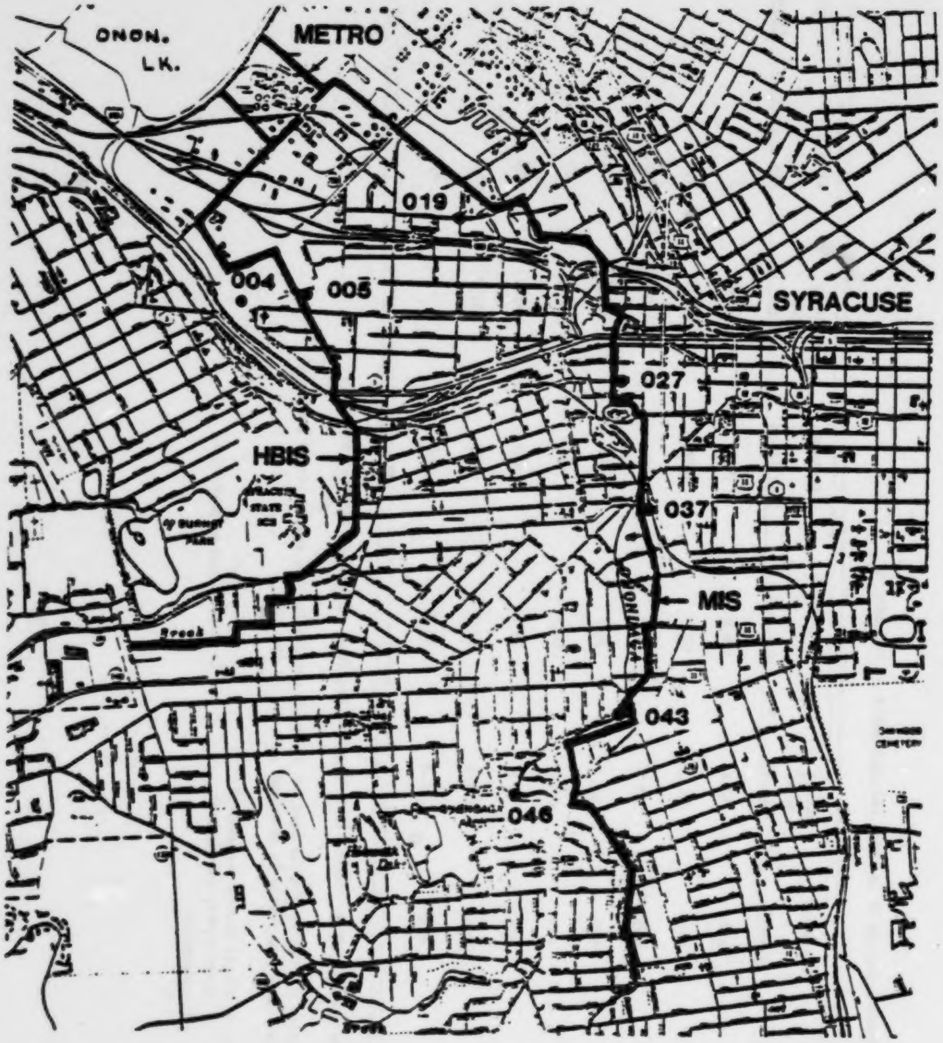

ONON.
LK.
METRO
019
004
005
SYRACUSE
027
HBIS
037
MIS
043
046

Figure 4. Location of Project CSO Sites

(c) available baseline data - physical and chemical analyses of CSOs in the Syracuse area have been performed during previous characterization studies. These data could serve as a baseline to which this study could be compared. However, this criterion was considered least important in site selection.

CSO samples were collected during four wet weather events in glass containers lowered from manholes directly above the overflow pipe. Sufficient sample was transferred to fill an amber glass bottle for Ames testing, a 50 ml plastic container with nitric acid preservative for TOC and heavy metals analysis, and a plastic half gallon container for other chemical/physical analyses. This sample storage and preservation procedure was followed for all samples. Glass containers were used for the collection and storage of Ames samples since it was unknown what effect plastic would have on the samples.

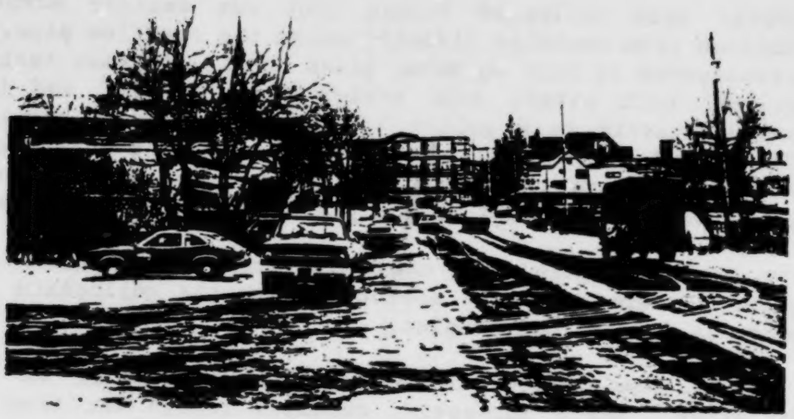
Sanitary sewage samples were collected from the combined sewer either before or after a storm event, as appropriate to the collection schedule, in the same manner as for the CSO samples.

Urban runoff samples were collected from catchbasins in their corresponding CSO sites. Where necessary, curbside runoff was used. Figures 5 through 8 depict the sample collection points.

Samples were collected from the north and south basins of Onondaga Lake at the 9 meter level, the lower limit of the epilimnion (44).

Rain samples were collected at the O'Brien & Gere Syracuse office in 36-inch diameter stainless steel pans pre-rinsed with DCM. These pans were placed away from buildings, cars, trees and pavement to minimize contamination from extraneous sources. Rain from two pans was combined until sufficient sample was collected for analytical purposes. The pH of this sample was taken immediately; the sample was then dispensed as previously described.

Additionally, three CSO samples were collected in Washington, D.C., one each at three separate sites. Due to difficulties in obtaining manual grab samples at these sites, collection was facilitated by the use of an automatic sampler. Also, a CSO and a runoff sample each were taken from a site in Rochester, NY (site 007-Maplewood Avenue) during two storm events (a total of two CSO and two runoff samples). Table 7 presents a characterization of all of these CSO sites.



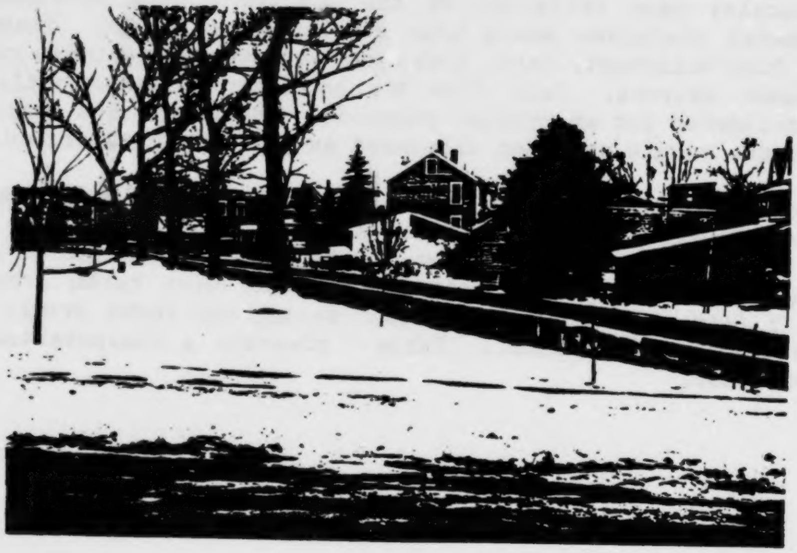
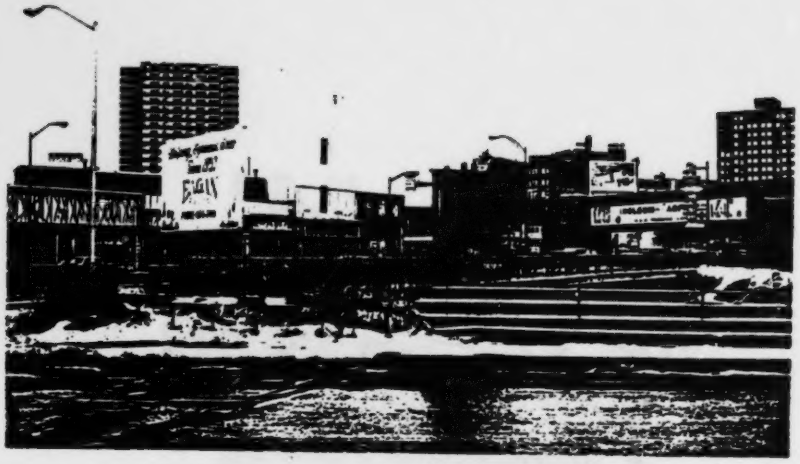


Figure 5. Sample Collection Sites: (above) Drainage Area 004, looking south; (below) Drainage Area 005, looking south - concrete encasement crossing center of picture contains Harbor Brook

Figure 6. Sample Collection Sites: (above) Drainage Area 005, looking west; (below) Drainage Area 019, looking north



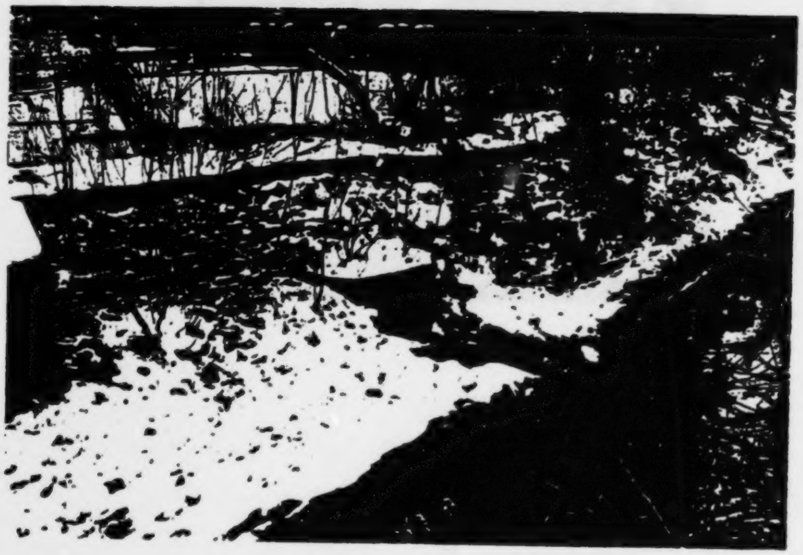


Figure 7. Sample Collection Sites: (above) Drainage Area 037, looking northeast; (below) Drainage Area 037, CSO discharge

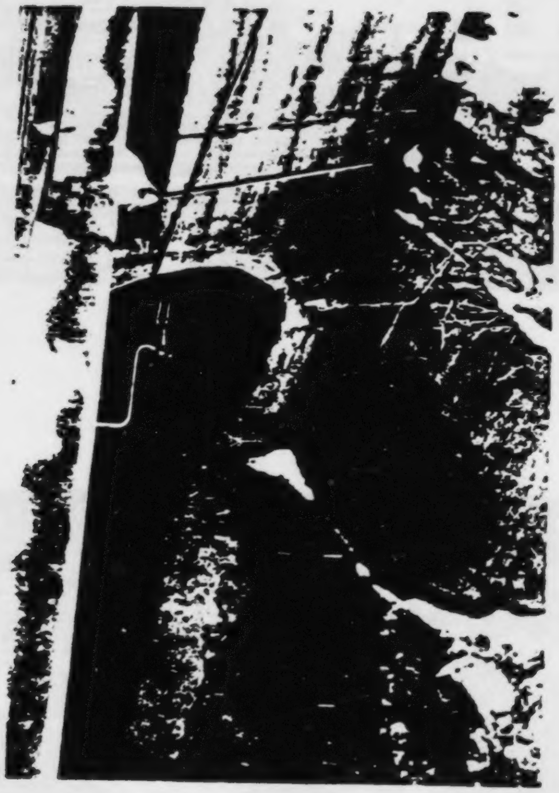


Figure 8. Sample Collection Sites: Drainage Area 043, CSO discharge

TABLE 7. CHARACTERIZATION OF CSO DRAINAGE AREAS IN ROCHESTER, NY AND WASHINGTON, D.C.

Site Location	Site Number	Area (Acres)	Percent Impervious	Land Use[a]
Rochester	007	729	38	1,2
Washington	024	4235	60	3,4
Washington	034	555	65	3,4
Washington	038	320	55	3,4

[a] land use:
1- single family residential
2- multi-family residential
3- commercial
4- industrial

Sample collection in Syracuse was performed by personnel from the Onondaga County Department of Drainage and Sanitation. Initially, the criterion described in the plan of study for frequency of wet weather sample collection was that storms would be sampled if they were separated by a four day interval from any previous wet weather event. In Syracuse, normally a rainy city, this was not considered to present a problem. However, the Northeast experienced an extremely dry year in 1980 and the first half of 1981, producing drought conditions in the region. This lack of precipitation, coupled with concern for the safety of sample collection personnel if it became necessary to work at night in highly trafficked streets, resulted in plans to reduce the sampling interval, if necessary. However, due to a continued lack of precipitation, wet weather samples were collected within 7 days of each other in five of six instances (Table 8). Sampling dates and times are presented in the tabulation in Appendix A. Rain data for the four storm events in 1980 and two in 1981 are presented in Table 8.

TABLE 8. RAIN DATA FOR STORM EVENTS, SYRACUSE, NY.

Sampling Date	Rainfall Start	Duration Stop	Volume (inches)	Antecedent Dry Days
7-29-80	0500	1400	0.87	2
9-17-80	1500	1700	0.27	2
10-13-80	0400	2200	0.07	5
10-20-80	1700	2400	0.12	2
9-2-81	0700	1000 (10-3-81)	0.83	8
9-4-81	0700	0300 (10-5-81)	0.40	1

Sample collection in Rochester, NY and Washington, D.C. was performed by O'Brien & Gere personnel. All samples were transported to the Syracuse laboratory by air as soon as practical, usually within 3 hours, where they were assigned a laboratory accession number. Sample portions for chemical/physical analysis were refrigerated; the portion for Ames testing was treated as discussed elsewhere in this section.

DATA MANAGEMENT

A computerized system for the identification of samples and management of data generated by any project has been developed. For this project, it included:

a. field identification - a field check sheet (Figure 9) was prepared for sample collection personnel which contained all pertinent identification information for any sample. A sheet was prepared for each sample and accompanied the appropriate sample containers to the laboratory.

b. sample identification - as each sample enters the laboratory, it was assigned a unique, five-digit accession number found on a sample identification ticket. This identifier permits the discrete organization of all information and data relating to that sample, whether for analytical identification purposes, reference in paper-copy records and correspondence, or complete storage and recall.

Temporary No. ____________
Sample Data ____________
Sample Time ____________

SAMPLE I.D. CHECKLIST

(1) SYRACUSE CSO & URBAN MUTAGENICITY STUDY

	2	3	4
1	☐ CSO	Site Number ____________	1 ☐ wet weather. 2 ☐ dry weather, before storm 3 ☐ dry weather, after storm
2	☐ Sanitary Flow	Site Number ____________	1 ☐ before storm 2 ☐ during storm 3 ☐ after storm
3	☐ Runoff	Site Number ____________	Comments:
4	☐ Metro	1 ☐ Influent 2 ☐ Effluent	1 ☐ wet weather 2 ☐ dry weather, before storm 3 ☐ dry weather, after storm

5 ☐ Onondaga Lake Depth ____________
6 ☐ Erie Boulevard Storm Sewer
7 ☐ Rain Comments:

Figure 9. Field Data Check Sheet.

c. data organization - in a preliminary planning phase of any analytical investigation involving the O'Brien & Gere laboratory, a computer codification format must be established which will later serve as the basis for data storage, recovery and identification. This format is characterized by the categorization of samples, with any type of identification permissible for the classification. The chosen coding scheme for identification having been established, the description of an individual sample may be recorded in appropriate identification fields on the sample ticket. There are seven distinct identification fields, four of which carry variable options and contain 9999 subcategories, while a fifth contains 999 subcategories. These permit several hundred options for the segregation of data and increase the ease of sorting edited data for evaluation.

For this study, a scheme was developed for the classification of project samples (Table 9). This scheme permitted the segregation of data by the city sampled and the sample source.

CHEMICAL ANALYSIS

All chemical analyses were performed by approved procedures (1,44). The pH of all samples was measured from an unpreserved aliquot of sample immediately upon arrival in the laboratory with a Corning Model 12 pH Meter (Corning Scientific Instruments, Corning, NY). TOC of nitric acid preserved samples was determined on a Beckman Model 915 Total Organic Carbon Analyzer (Beckman Scientific Products, Fullerton, CA). Heavy metal analysis was performed on a Varian AA-575 Series Atomic Absorption Spectrophotometer (Varian Techtron, Springvale, Australia).

MUTAGENICITY TESTING

General

As mentioned previously, three sources served as the primary references for mutagenicity testing procedures (3,5,57). The procedures used were largely those of Robnett (57); modifications from other sources were subject to review and approval by the EPA. Some additional modifications were suggested by the EPA (19,65) and were incorporated into the project; they are discussed as appropriate.

Sample Preservation and Disinfection

One of the limitations of the Ames test is the requirement that samples be sterile prior to their application in the test. Besides the obvious fact that any bacterial contamination would interfere with the growth and scoring (counting) of colonies produced by the test strains, metabolic by-products from microbial contaminants could interact with the test substance to produce false results.

Since samples might arrive in the laboratory at any time of the day or night, and immediate extraction would be impractical, it was necessary to develop a procedure to disinfect samples upon their arrival to reduce

TABLE 9. PROJECT SAMPLE AND DATA MANAGEMENT IDENTIFICATION SCHEME.

ID#1 = City	ID#2 = Sample Source	ID#3 = Location	Sample ID #4 = Descriptor
1 = Syracuse	1 = CSO	Site Number	1 = wet weather 2 = dry weather, before storm 3 = dry weather, after storm
	2 = Sanitary Flow	Site Number	1 = before storm 2 = during storm 3 = after storm
	3 = Runoff	Site Number	
	4 = Metro	1 = influent	1 = wet weather 2 = dry weather, before storm 3 = dry weather, after storm
		2 = effluent	1 = wet weather 2 = dry weather, before storm 3 = dry weather, after storm
	5 = Onondaga Lake	1 = south basin 2 = north basin	Depth (meters)
	6 = Erie Boulevard Storm Sewer		
	7 = Rain		

TABLE 9. PROJECT SAMPLE AND DATA MANAGEMENT IDENTIFICATION SCHEME.

ID#1 = City	ID#2 = Sample Source	ID#3 = Location	Sample ID #4 = Descriptor
2 = Washington, D.C.	1 = CSO	Site Number	1 = wet weather 2 = dry weather, before storm
	2 = Sanitary Flow	Site Number	1 = before storm
	3 = Runoff	Site Number	
3 = Utica, NY	1 = CSO	Site Number	1 = wet weather 2 = dry weather, before storm
	2 = Sanitary Flow	Site Number	1 = before storm
	3 = Runoff	Site Number	
4 = Rochester, NY	1 = CSO	Site Number	1 = wet weather 2 = dry weather, before storm
	2 = Sanitary Flow	Site Number	1 = before storm
	3 = Runoff	Site Number	

microbial degradation of substances in the sample over time. Normal oxidant disinfectants (chlorine, chlorine dioxide, other halogen compounds, ozone) are known to alter chemical species, causing the formation of mutagenic compounds from non-mutagens; autoclaving the sample could destroy heat labile (sensitive) organic mutagens, or cause their volatilization; filtration could remove mutagenic organics adsorbed to particulates or oil and grease globules; and irradiation with ultra-violet might alter the structure of target compounds, since many known organic carcinogens and mutagens are UV-absorptive. Therefore, an alternative was required which would not physically or chemically alter the compounds present in the environmental sample.

Overbye and Margolin (50) used 1.0 ml of chloroform to disinfect 250 ml of raw water sample from the Arthur Kill (New York City). Since chloroform, a known carcinogen, is one of the few carcinogenic substances which is not mutagenic in the Ames test (62), its presence could not cause any false positive results. Chloroform, heavier than water, would sink in globules to the bottom of the sample container after agitation and disinfection. Aliquots of disinfected, raw sample could then be removed for testing from the top of the sample container without removing any of the chloroform disinfectant.

The chloroform procedure was evaluated for use in this study. Samples of wastewater influent to the Metropolitan Syracuse Treatment Plant were collected; these were used in the disinfection evaluation and considered to be representative of the most microbially contaminated environmental sample likely to be encountered during the project. However, the chloroform disinfection procedure was found not to be of value for three reasons:

a. at the ratio of chloroform suggested (50) in 100 ml samples, disinfection was not achieved. The concentration of chloroform was increased without improved results. Samples containing varying amounts of chloroform were shaken at 0, 10 and 20 minutes, then allowed to settle. Aliquots of 1.0 ml were removed from the top of each sample container, spread plate inoculated onto ONA plates and incubated at 37°C for 24 hours. Disinfection was not achieved. No specific reason for this lack of disinfection efficiency is evident, nor was further investigation of the subject conducted.

b. spot tests performed on some raw test samples did not elicit positive results, indicating a lack of mutagenicity of the raw sample. An explanation of the spot test, a rapid screening method for mutagenicity, is presented in a following section.

c. chloroform might be acting as an extractant in the disinfection procedure, thereby removing mutagenic organics from the aqueous layer. These organics would then be contained in the heavier chloroform phase at the bottom of the flask. This process could have resulted in a false negative spot test result for test samples when test sample was removed from the upper aqueous phase in the flask. Since the efficiency of chloroform as an extractant in this test has not been demonstrated, the recommended extractant, dichloromethane (57), was used. Extensive testing, beyond the scope of this project, would have been required to validate chloroform as a suitable extractant.

The EPA recommended extraction procedure (57)(44 FR 69541) involves the use of dichloromethane (methylene chloride). DCM was evaluated as a disinfectant in the same manner as chloroform; the results were found to be favorable.

The procedure used was the addition of 100 ml of DCM to 2 l of sample in the glass sample container. The sample was shaken vigorously for two minutes. This served to disinfect the sample and provide the first of three extractions. The sample bottle was vented to release vapor pressure, then recapped.

Extraction and Concentration

The extraction and concentration procedure used for this project was that recommended by the EPA (57) with minor modifications. The extraction procedure was as follows:

a. (if the sample were neutralized and disinfected as described in the preceding section, this procedure could proceed directly to step (c).) The sample was shaken well; 2 l was measured into a separatory funnel following neutralization with either 6N NaOH or 6N HCl, as applicable. Generally, neutralization was not required since most samples were close to pH 7.

b. 60 ml of DCM was added first to the graduated cylinder used to measure the sample, swirled to rinse the sides, then transferred to the sample bottle; it was capped and shaken 30 seconds to rinse the bottle walls. This solvent was transferred to the separatory funnel.

c. the funnel was shaken for 2 minutes with periodic venting to release vapor pressure. The organic (bottom) layer was allowed to separate for a minimum of 10 minutes. The DCM layer was collected in a 250 ml flask; any emulsion was reserved in the separatory funnel with the aqueous phase.

d. steps (b) and (c) were repeated an additional two times, with the extract being collected in the same flask each time. Following the third extraction, any remaining emulsion was taken into the flask.

e. the combined extract in the flask was dried with anhydrous sodium sulfate, (Na_2SO_4), adding sufficient quantities for the salt to flow freely. Since the emulsions formed generally were extensive and would have clogged a Na_2SO_4 column, but may have contained target compounds, the salt was added directly to the flask containing the combined extract.

f. the extract was transferred into a 500 ml Kuderna Danish flask. The drying flask was rinsed with three 5 ml aliquots of DCM, each rinse being combined with the extract in the Kuderna Danish flask.

g. the Kuderna Danish flask was topped by a Snyder column and the extract reduced to 1-5 ml in a water bath.

h. the extract was transferred to a 1.0 ml graduated concentration tube and placed in a Kontes Tube Heater (Kontes Scientific, Vineland, NJ). As the volume was reduced, any remaining extract was added to the tube; the extract was not permitted to reduce to dryness. Finally, the 250 ml flask was rinsed three times, each with 1-2 ml of DCM; these rinses were added to the concentration tube and reduced in the same manner. The nitrogen (N_2) blowdown step in the recommended procedure (57) was not employed since it was found that the 2-step Kuderna Danish/tube heater concentration readily reduced the extract to the desired volume. The additional glassware transfer which the N_2 step may have required could have increased the risk of contamination or loss of sample.

i. the remaining extract was transferred to a storage vial. The graduated tube was rinsed three times with DMSO, and brought to a final volume in the vial of 10 ml. This resulted in an effective concentration factor for this extract of 200 times over the original 2 liter sample. The resultant extracts are depicted in Figure 10.

A water blank and two spiked water samples were included in each batch of sample extracted. One spike was a mutagen active without microsomal activation, the other with activation.

Spot Tests

Spot tests (5) were performed on the first 32 samples extracted (25 samples, 3 blanks, 4 spikes) using three of the test strains (TA98, TA100, TA1537). The spot test is the simplest method of testing compounds for mutagenicity and may be used for the rapid screening of large numbers of compounds in a short period of time. A drop of test compound is placed directly on the agar surface. As the compound diffuses through the medium, an indication of mutagenicity, toxicity, and necessity of metabolic activation can be obtained. In this instance, aliquots of 32 of the test samples were applied as approximately 50 ul of the raw concentrate (2 pipet drops). Plates were incubated for 48 hours at 37°C. Of these samples, sixteen were toxic to one or more of the test strains (Table 10), with 12 samples toxic to TA98, six samples toxic to TA100, and only three toxic to TA 1537. However, none of these samples exhibited any positive mutagenicity.

Due to the difficulty of obtaining any meaningful preliminary results using the spot test with these samples, it was dropped from the procedure.

Figure 10. Concentrated Sample Extracts

TABLE 10. RESULTS OF SPOT TESTS: SAMPLE TOXICITY (+ indicates toxicity).

Sample Number	TA 98	TA 100	TA 1537
94251	+		
94252	+		
94253	+	+	
94254		+	
94255	+		
94256	+		
94482			
94490		+	+
94491		+	
94492	+	+	
94493	+		
94494	+		
94495			
94496			
94497			
94498			
94499			
94500			
94501			
94507	+		+
94508			
94635			
94636			+
94660	+		
94661	+	+	
94662			
94663	+		
94765			
94766			
94780			
94781			
94782			

Ames Testing

Detailed procedural descriptions of the Ames test are available elsewhere (3,5,57). This section will only highlight the procedures used in this study.

For Phase I, two 10-fold serial dilutions were prepared from the sample extract/concentrate, using sterile distilled, deionized water as the diluent. These dilutions resulted in three concentrations (57) for application in the test: 200X (the extract), 20X (the first 10-fold dilution), and 2X (the second 10-fold serial dilution). A dosage of 100 ul of each of these concentrations was used, each applied in duplicate. The 100 ul dosage of the most concentrated form of the sample was the strongest application used for the determination of a positive response; this can be defined as representing the analysis and evaluation of the worst case available in the form of a highly concentrated sample extract. Initial attempts with the application of 500 ul of sample resulted in toxicity to test strains in too many cases for general applicability.

A 100 ul portion of the appropriate tester strain was added to a test tube containing 3.0 ml of melted top agar, followed by sample. A 500 ul aliquot of the S-9 reaction mixture was added last, as appropriate, just prior to mixing of the tube's contents and pouring them into the petri plate containing minimal medium.

A preliminary evaluation of the methodology indicated some difficulty in spreading the sample-top agar mixture evenly over the medium in the petri dish due to the quality of the concentrated sample. Therefore, the amount of top agar was increased from the recommended 2.0 ml (5,57) to 3.0 ml; the concentration of histidine and biotin in the top agar was kept the same. It was expected that this increase in histidine-biotin/plate might result in an increase in spontaneous revertants due to the availability of a greater amount of histidine and biotin per plate. However, by using this method for all controls, internal consistency is maintained. Spontaneous revertant levels are available elsewhere in this section. They demonstrate the improved spontaneous revertant levels obtained with this procedure.

Figure 11 presents the full range of concentrations and dosages used for each sample during Phase I; concentrations and dosages used during Phase II will be described in subsequent sections.

Plates were wrapped in plastic bags prior to incubation. This reduced moisture loss. Also, the risk of contaminating surrounding plates from those of one sample which may contain a volatile mutagen was reduced. However, the *Salmonella* strains generally do not respond to volatile compounds unless the tests are conducted individually in sealed desiccators (62).

The plates were incubated at 37°C for 48 hours; revertant colonies were then counted on a Darkfield Quebec Colony Counter (American Instrument Co., Silver Spring, MD). All plates were reviewed under a Bausch & Lomb Dissecting Stereoscope (20X) (Bausch & Lomb, Rochester, NY) to detect any possible effect of the sample on the level of background growth.

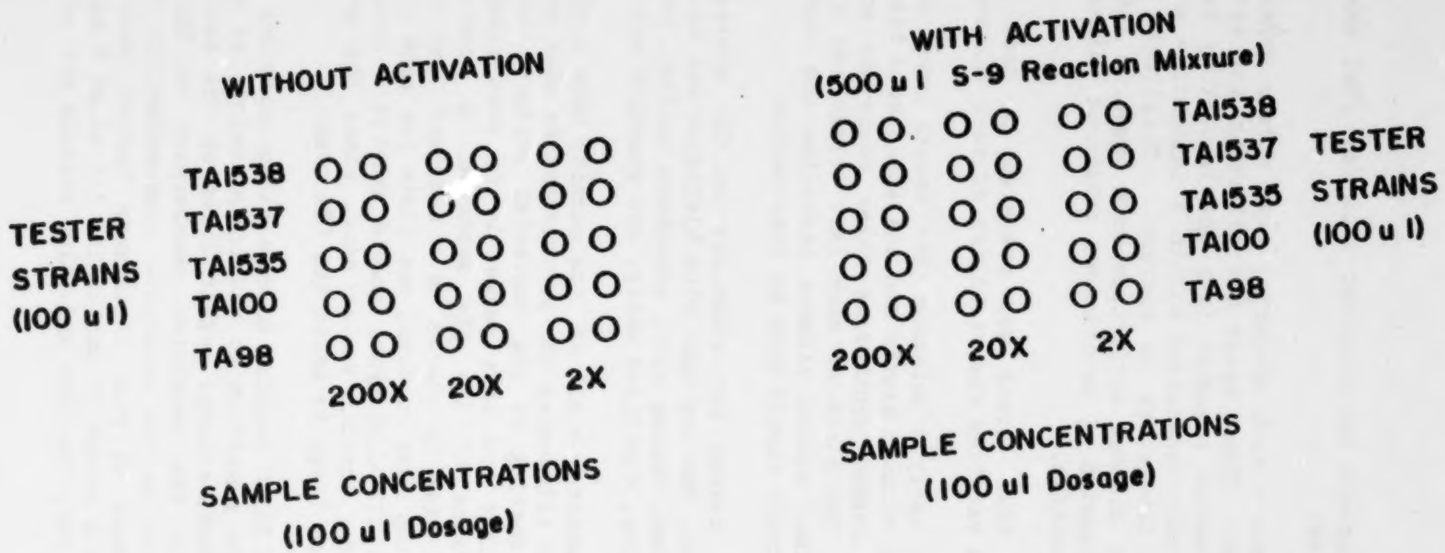

WITHOUT ACTIVATION
TESTER
STRAINS
(100 u l)
TA1538
TA1537
TA1535
TA100
TA98
200X
20X
2X
SAMPLE CONCENTRATIONS
(100 u l Dosage)
WITH ACTIVATION
(500 u l S-9 Reaction Mixture)
TA1538
TA1537
TA1535
TA100
TA98
TESTER
STRAINS
(100 u l)
200X
20X
2X
SAMPLE CONCENTRATIONS
(100 ul Dosage)

Figure 11. Range of Sample Test Concentrations and Dosages.

Controls

An extensive range of controls was prepared each day that samples were analyzed (5,57). These included:

a. spontaneous revertants - each bacterial test strain undergoes some spontaneous reversion. These rates are different for each strain. Although this rate varies somewhat from one laboratory to another, it should be relatively consistent within a laboratory and roughly comparable from one laboratory to another. Triplicate plates of each strain with and without activation were prepared for each day of analyses. A discussion of spontaneous revertant values for this study follows this section.

b. one plate of each test strain was prepared to test growth on nutrient agar. This verifies the viability of each culture.

c. The test strains all carry a deletion that covers genes involved in the synthesis of the vitamin biotin and the amino acid histidine, so that the organisms cannot synthesize these compounds and require them in the medium. One plate of each test strain was prepared to test growth on minimal medium without histidine and biotin. Only the wild type LT2 strain should grow on this medium.

d. all solutions were tested for sterility on ONA plates. These included the top agar, the top agar with histidine and biotin, DMSO, nutrient agar, minimal medium agar, phosphate buffer, S-9 reaction mixture, salt solution, distilled water, and standard solutions.

e. deep rough (rfa) character - all of the strains have a rfa, or deep rough, mutation that eliminates the polysaccharide side chain of the lipopolysaccharide coating of the bacterial surface, resulting in bacterial strains that are more permeable to test chemicals. To test for the presence of the rfa mutation, a crystal violet--impregnated filter disk is placed on a nutrient agar plate which already contains a top agar culture, one plate for each strain. All strains, except the LT2 wild type, should exhibit a zone of growth inhibition around the crystal violet disc, since the molecules of the compound are too large to enter the LT2 coat.

f. R factor - TA98 and TA100 contain an ampicillin-resistant (R) factor that makes them more sensitive to chemicals, mainly as a result of an error prone recombinational repair locus on the bacterial DNA. TA98 (R factor) is the sensitive counterpart to TA1538 (no R factor), while TA100 is the sensitive counterpart to TA1535. To test for the presence of this resistance factor, each strain is cross-streaked over a streak of ampicillin (10 ul of 8 mg/ml in 0.02 N NaOH) solution. Only the TA98 and TA100 strains are not inhibited by the ampicillin.

Each of these aforementioned steps was taken each day that analyses were performed. In addition, other quality control procedures were required. These were:

a. extraction blanks - when a group of samples is prepared for extraction, a blank consisting of 2 l of distilled, deionized water is extracted concurrently. Preliminary investigations for this study have indicated that this blank should not provoke a positive response in the test.

b. extraction spikes - standard solutions of each of two mutagens is added to two respective 2 l portions of distilled, deionized water. One mutagen is a compound active without metabolic activation, while the second is active upon activation with the S-9 liver preparation. These spikes are extracted concurrently with each group of sample extractions. Initially, MNNG was used as the unactivated compound, although this was changed to MMS (varying concentrations from 100 ul of a 10 ul/ml solution to 100 ml of MMS in a 2 l blank), when a problem was perceived in the analytical detection of the MNNG in the Ames test following extraction. The activated compound was BaP (varying concentrations from 100 to 500 ul of a 1000 ug/ml solution).

c. positive controls - positive controls are used to test the bacterial strains for mutagenic properties. This test must be performed each time the strains are used. The same compounds used as extraction spikes were used as positive controls.

d. duplicates - two samples, one each during the Phase I and Phase II analyses, were split. Equivalent aliquots were extracted, concentrated and assayed at the O'Brien & Gere laboratory and that of EPA Region II Environmental Services Division, Edison, New Jersey. Sample portions were transported to the EPA in refrigerated containers. A comparison of the split samples is presented in Section 7 (Results).

Spontaneous Revertants

Spontaneous reversion rates are affected by the environment in which the strains are grown. Routine factors such as differences in brand and batch compositon of media, atmospheric contaminants and brands of petri plates may cause interlaboratory variations. Internal consistency may be affected by soft agar layer thickness and evenness, temperature uniformity during incubation, incubation interval, consistency of plate agar volume, interactions of mutagens and S-9, mixing technique, and storage and controls for tester strains (69).

Column I of Table 11 presents the spontaneous reversion values obtained for each of the five tester strains obtained from Dr. Ames by the O'Brien & Gere laboratory while Column II lists the range of values reported in his original methods paper (5). The values in Column I are the arithmetic means

TABLE 11. COMPARISON OF SPONTANEOUS REVERSION RATES FOR BACTERIAL TEST STRAINS.

Test Strain	I	II	III	IV	V	VI	VII
TA98	21	30-50	61	63	23	45	15-75
TA100	65	120-200	305	306	146	178	60-220
TA1535	12	10-35	26	47	16	27	5-50
TA1537	4	3-15	10	14	7	12	3-25
TA1538	6	15-35	30	31	5	24	5-40

I - O'Brien & Gere historical values.
II - Ames, McCann, and Yamasaki, 1975 (5).
III - EPA, Edison; 7-27-81.
IV - EPA, Edison; 8-6-81.
V - EPA cultures, O'Brien & Gere laboratory.
VI - EPA cultures, O'Brien & Gere laboratory, 120% biotin-histidine.
VII - de Serres and Shelby, 1979 (15).

of such revertants compiled from historical performance in the O'Brien & Gere Laboratory and are based on a minimum of 30 replicates. Based on these values, the revertant rates used in this study may have been somewhat low, although they did exhibit internal consistency. For this reason, the cultures were used for all Phase I analyses.

As a result of these values, however, the potential may have existed for some loss of sensitivity in the analytical results. Therefore, a second phase of the study was initiated at the request of the project officer. Samples were collected from two drainage areas which had exhibited detectable responses in Phase I analysis. Copies of the five Salmonella tester strains were obtained from the EPA Region II Environmental Services Laboratory at Edison, NJ. Spontaneous revertant values obtained from these cultures by EPA-Edison are presented in Columns III and IV of Table 11, while Column V represents values obtained for these cultures in the O'Brien & Gere laboratory. Since the Column V values were lower than the EPA values, the concentrations of the histidine and biotin solutions were increased to 120%, at the suggestion of the EPA-Edison lab, on the basis that autoclave sterilization might be causing a breakdown of one of the two components. The resultant spontaneous revertant values (Column VI) are more comparable to those obtained at EPA-Edison.

de Serres and Shelby (15) reported the spontaneous revertant values obtained from eight laboratories (Column VII). Based on these values, the spontaneous revertant values for the tester strains used in Phase I, while in the low range, all are within a range considered acceptable. The authors caution that although the reported range is wide, the range for any tester strain in one laboratory is narrower and consistent; these criteria should govern acceptability of spontaneous revertant values.

SECTION 7

RESULTS

CHEMICAL ANALYSES

Tables 12 through 14 summarize the chemical data obtained during this study. A detailed tabulation of all chemical data is available in Appendix A.

Although the number of urban runoff samples collected in Syracuse was limited to sixteen, a wide sample-to-sample variability is evident (Table 12). It is also interesting to note that a comparison of the limited data from each site finds values within the same approximate range. Rain analyses summarized on the same table resulted in extremely low levels of the parameters measured, including a low pH range. Additional pH measurements obtained from precipitation in Syracuse (O'Brien & Gere Engineers, Inc., unpublished data) indicates that this low range of pH values is not unusual, indicating a local acid precipitation problem.

Results of the chemical analysis of CSO (Table 13) and sanitary sewage samples (Table 14) collected in this study are comparable to those found in previous studies performed in Syracuse (48). Table 13 contains data obtained from the chemical analysis of CSO during this study from samples collected concurrently with those for mutagenicity, as well as data obtained from the same sites during CSO characterization studies of 1976-1977 (in parentheses). In urban runoff, BOD values were generally lower than those in CSO and sanitary waste, TSS was higher than in either CSO or sanitary samples, and PB concentrations were higher than in either of the other sample sources. O&G values were generally higher in samples of sanitary waste than in CSO or runoff samples.

MUTAGENICITY TESTING

Phase I

Criteria used in this study for the determination of a significant level of mutagenicity were recommended by the EPA (19,65). The determination of significance for each strain is based on the formula

$$MAR = \frac{E-C}{c}$$

where

MAR = mutagenic activity ratio
E = the number of induced revertants

TABLE 12. SUMMARY OF CHEMICAL ANALYSES FOR URBAN RUNOFF AND RAIN, Values in mg/l Unless Otherwise Noted.

Site Number	pH Range	BOD_5	TOC	TSS	CD	CR	PB	CU	O&G
4	6.8-7.2	35	80	370	0.01	0.03	0.88	0.44	72
19	6.1-8.3	16	90	750	0.01	0.03	0.74	0.16	61
27	6.2-8.9	14	52	213	<0.01	0.01	0.62	0.09	55
37	6.8-8.0	30	87	663	<0.01	0.01	0.84	0.14	102
43	7.0-7.9	40	81	807	<0.01	0.02	0.96	0.12	168
46[a]	7.3	20	49	760	<0.01	< 0.01	0.53	0.03	90
All[b]	6.1-6.9	26±17	76±37	570±320	<0.01	0.02±0.01	0.79±0.58	0.18±0.30	80±69
Rochester-Site	7.3	32	29	290	<0.01	< 0.01	0.43	0.05	70
Rain	3.8-5.5	3	4	2	< 0.01	< 0.01	< 0.01	0.05	-

[a] - only one sample available

[b] ± standard deviation

TABLE 13. SUMMARY OF CHEMICAL ANALYSES FOR CSO SAMPLES[a], Values in mg/l Unless Otherwise Noted.

Site Number	pH Range	BOD_5	TOC	TSS	CD	CR	PB	CU	O&G
4	6.5-7.3 (6.1-7.5)	50 (82)	71 (121)	123 (530)	<0.01 (0.01)	< 0.01 (0.005)	0.17 (0.01)	0.10 (0.024)	30 (-)
19	6.8-9.1 (-)	48 (-)	71 (-)	392 (-)	0.01 (-)	0.02 (-)	0.29 (-)	0.14 (-)	62 (-)
27	3.7-7.5 (5.5-7.4)	32 (127)	40 (128)	143 (450)	<0.01 (0.01)	0.06 (0.042)	0.14 (0.065)	0.26 (0.050)	23 (33)
37	6.4-7.1 (7.0-7.6)	116 (22)	59 (38)	163 (58)	<0.01 (-)	< 0.01 (0.016)	0.06 (-)	0.10 (0.035)	74 (-)
43	7.0-7.2 (6.8-10.2)	145 (107)	102 (111)	355 (509)	<0.01 (-)	0.36 (0.022)	0.24 (-)	0.37 (0.060)	85 (-)
46	7.4[b] (6.9-7.9)	10 (151)	65 (161)	1070 (444)	<0.01 (-)	< 0.01 (0.010)	0.45 (-)	0.04 (0.031)	100 (-)
All[c]	3.7-9.1	70±61	84±87	275±262	<0.01	0.09±0.29	0.19±0.25	0.19±0.24	55±35
Washington, DC (All)	6.5-6.8	27	35	77	<0.01	< 0.01	0.06	0.04	68
Rochester	7.1	6	24	8	<0.01	<0.01	<0.01	0.01	90

a - historical data in parentheses.

b - only one sample available from this study.

c - all data from this study only; ± standard deviation.

TABLE 14. SUMMARY OF CHEMICAL ANALYSES FOR DRY WEATHER SANITARY SAMPLES, Values in mg/l Unless Otherwise Noted

Site Number	pH Range	BOD_5	TOC	TSS	CD	CR	PB	CU	O&G
4	5.3-7.5	182	118	95	< 0.01	0.04	< 0.01	0.08	123
19	6.8-8.8	137	76	143	0.01	< 0.01	< 0.01	0.08	68
27	7.4-7.7	58	10	10	< 0.01	< 0.01	< 0.01	0.03	31
37	6.1-7.0	322	145	206	< 0.01	< 0.01	0.06	0.07	317
43	6.8-7.4	137	58	210	< 0.01	0.05	< 0.01	0.09	73
46[a]	6.9	50	22	70	< 0.01	< 0.01	< 0.01	0.04	100
All[b]	5.3-8.8	162±140	82±98	125±112	< 0.01	0.02±0.03	< 0.01	0.07±0.03	121±132

[a] - only one sample available.
[b] - ± standard deviation.

C = the number of spontaneous revertants on the day that the E revertants were induced

c = the historical rate of spontaneous reversions in the testing laboratory.

MAR values were calculated for all samples, with all five test strains, without and with S-9 liver activation. These are tabulated and presented in Appendix B. A negative MAR results when E is less than C for a given test strain on a particular day; a zero value is obtained when E equals C, and a positive value results from E greater than C, although this positive value may only be a fraction if E is only slightly greater than C. A sample is considered positive if it has a MAR of 2.5 or greater.

Table 15 summarizes only those samples and the particular strains(s) with a MAR of 2.5 or greater. The MAR values presented are for 100 ul of a 200X sample concentrate (the highest concentration of any sample available in this study and, therefore, the most likely sample dosage to induce mutagenic activity), with 500 ul of S-9 reaction mixture and 100 ul of the appropriate test culture. Nineteen samples induced a detectable response in one or more of the test strains by this criterion.

Nine of these nineteen samples which produced detectable responses were of urban runoff, seven collected from Syracuse and two from Rochester, NY, or 47% of the runoff samples collected, while five of the samples which produced detectable responses (5 of 19, or 26%) were CSO, three from Onondaga County and two from Washington, D.C.

Nine of the various samples listed in Table 15 as inducing a positive MAR were mutagenic in TA1538 yet not in the more sensitive counterpart, TA98. There have been indications that this is not an unusual test result, that TA1538 may show greater sensitivity than TA98 with some chemicals (15).

No MAR of 2.5 or greater was obtained from the analysis of CSO from sites 4 and 46; sanitary wastewater from sites 37, 43, and 46; urban runoff from sites 27 and 46; influent to the Metropolitan Treatment Plant; or CSO samples from Rochester, N.Y.

Split Sample Analyses

During Phase I of the study, a sufficient volume of OBG sample number 95864 Syracuse CSO from site 004, was collected so that one gallon could be forwarded to the laboratory of the USEPA Region II Environmental Services Division in Edison, NJ for concurrent analysis. The laboratory procedure used is summarized in Appendix C.

TABLE 15. SUMMARY TABLE OF PHASE I MUTAGENICITY RESULTS.

O'Brien & Gere Number	ID CODE City	ID CODE Source	ID CODE Location	ID CODE Descriptor	MAR[a] TA98 Without S-9	MAR[a] TA98 With S-9	MAR TA100 Without S-9	MAR TA100 With S-9	MAR TA1535 Without S-9	MAR TA1535 With S-9	MAR TA1537 Without S-9	MAR TA1537 With S-9	MAR TA1538 Without S-9	MAR TA1538 With S-9
94492	1	1	19	1		2.9	2.8							
95366	1	1	27	1										7.2
95368	1	1	43	1										9.1
95475	1	2	4	2										2.7
95476	1	2	19	2										3.9
95477	1	2	27	2				11.2						5.1
94491	1	3	4	1		3.3					4.1	2.6	3.4	2.5
95739	1	3	4	1	3.0	2.6					4.0	4.8		
94493	1	3	19	1					2.6					
94497	1	3	37	1	2.7									
95745	1	3	37	1										2.9
95871	1	3	37	1			6.8	2.9						
95873	1	3	43	1										10.0
95474	1	4	2	2										6.1
95369	1	7												2.6
94765	2	1	24	1									4.4	
94766	2	1	34	1			16.2				4.2			
94662	4	3	7	1									4.5	
94663	4	3	7	1									3.8	

[a] MAR - mutagenic activity ratio

The Ames test was performed by the EPA on the first sample aliquot using the Region II extraction procedure (57) (Appendix C). The test was conducted, with and without metabolic activation, using five volumes of extract (1, 10, 100, 250, 500 ul), each in duplicate. The results of the test indicated a positive mutagenic response TA1537 (pH 12 extract without metabolic activation at 500 ul (MAR = 4.8). The experiment was repeated using six volumes of extract (100, 200, 300, 400, 500, 600, ul), each in duplicate. The results of this test indicated a positive mutagenic response with the bacterial tester strains TA1537 and TA1538 (pH 12 extract) without metabolic activation at 600 ul (TA1537, MAR = 2.9; TA1538, MAR = 5.0).

A second aliquot of the sample was extracted on the seventh day after receipt, using the EPA Region V procedure (57). However, the Region V criteria specifies that the sample should be extracted within the seven days following collection. The test was conducted, with and without metabolic activation, using five volumes of extract (100, 200, 300, 400, 500 ul), each in duplicate. The results of the test indicated a negative mutagenic response with the five bacterial tester strains. Possibly the results differed from those obtained with the Region II criteria because the sample was held over the recommended storage period.

The second aliquot was assayed at the O'Brien & Gere Laboratory using the analytical procedure discussed earlier. The test was conducted with and without metabolic activation, using 100 ul of extract at three concentrations (2X, 20X, 200X), each in duplicate. The results of the test were negative for all five bacterial tester strains, the same conclusion as that of the EPA laboratory.

Phase II

During Phase II, the verification phase of the study, a similar split sample was collected - O'Brien & Gere Engineers, Inc. (OBG) sample number 29031, wet weather influent to Metro. The EPA laboratory conducted the analysis by the Region V procedure as described in the preceding paragraphs but only at ambient pH. A detectable response was found for the test strain TA1538 without metabolic activation at 500 ul (MAR = 2.9). Repetition of the experiment resulted in an apparent increased response as follows:

Volume (ul)	MAR
400	25.8
450	36.6
500	41.9
550	7.2
600	-

No explanation for this apparent increase in mutagenicity is readily evident.

For the same sample, an MAR of 6.1 was obtained for TA1538 with 500 ml of the extract, and without metabolic activation. Upon repetition of the test, an MAR of 2.4 was found from the application of 500 ul of sample. Therefore, initially the results of the O'Brien & Gere laboratory did indicate a detectable mutagenic response in the split sample, as did the EPA lab; however, this response could not be confirmed, while that found by EPA-Edison was. The initial responses obtained by both O'Brien & Gere (MAR = 6.1) and that of EPA (MAR = 2.9) from 500 ul/plate applied dosage are of similar magnitude.

SECTION 8

DISCUSSION

INTERPRETATION OF RESULTS

To evaluate the data produced during this study, the results must be compared with the mutagenic responses of known mutagens or carcinogens. An inference of relative risk can then be made for the test sample. Unfortunately, such a comparison with other data reported in the literature is often not possible. Results may be reported in several forms:

(a) as revertants induced by test samples;

(b) as revertants induced by test samples, less the spontaneous revertants for each bacterial test strain;

(c) with the dosage of test sample/plate as umoles/plate or mg/plate; or

(d) as raw analytical data, or as a calculated sample/control ratio.

It has been recommended that all journal articles contain tabulated revertant values in reports of the results of studies (15). This may be impractical where publishing costs are critical and page space is at a premium. The use of the mutagenic activity ratio (MAR) offers a means of normalizing laboratory-to-laboratory variation in spontaneous revision levels while providing a stable index with which to compare results.

Phase I

The results of all applied dosages for samples which elicited a detectable MAR (equal to or greater than 2.5) are presented in Table 16. Three concentrations of 2X, 20X and 200X were applied at a dosage of 100 ul/plate at each concentration. Nine of the nineteen samples which produced detectable responses (a positive MAR) (Table 16) did so across the range of all three concentrations; six of these responses resulted from metabolic activation. Four of these nine samples produced an MAR of 2.5 or greater for all three concentrations; these four responses are presented in Figures 12-15. The four curves produced from the responses of these samples are generally horizontal with little slope, indicating that these samples did not exhibit an increase in the mutagenic response with an increase in the amount of sample applied. Generally, a response which increases with dosage or concentration would be expected; however, a response such as that exhibited by these samples is not unusual. The remaining five responses are presented in Figures 16-20. Only sample number 95871 (Figure 20) exhibited a logarithmic response curve.

TABLE 16. SUMMARY OF DETECTABLE RESPONSES, PHASE I ANALYSES.

Sample Number	Organism	S-9 Reaction Mixture (ul)	MAR[a]		
			200X[b]	20X	2X
94492	TA98	500	2.9	0.5	-
94492	TA100	-	2.8	0.3	-
95366	TA1538	500	7.2	3.2	1.2
95368	TA1538	500	9.1	6.0	2.3
95475	TA1538	500	2.7	2.8	3.4
95476	TA1538	500	3.9	4.0	4.0
95477	TA100	500	11.2	0.1	-
95477	TA1538	500	5.1	4.6	5.4
94491	TA98	500	3.2	0.2	-
94491	TA1537	-	4.1	2.5	-0.7
94491	TA1537	500	2.6	0.9	-
94491	TA1538	-	3.4	2.8	0.2
94491	TA1538	500	2.5	0.7	-
95739	TA98	-	3.0	1.1	-
95739	TA98	500	2.6	0.6	-
95739	TA1537	-	4.0	3.2	0.8
95739	TA1537	500	4.8	1.9	-
94493	TA1535	-	2.6	-0.1	-
94497	TA98	-	2.7	0.3	-
95745	TA1538	500	2.9	-0.7	-
95871	TA100	-	68.	12.	1.7
95871	TA100	500	29.	1.7	-
95873	TA1538	500	10.	2.1	-
95474	TA1538	500	6.1	5.6	6.5
95369	TA1538	500	2.6	1.3	-
94765	TA1538	-	4.4	0.8	-
94766	TA100	-	16.2	-0.5	-
94766	TA1537	-	4.2	-0.5	-
94662	TA1538	-	4.5	0.6	-
94663	TA1538	-	3.8	0.6	-

[a] - MAR = Mutagenic Activity Ratio

[b] - concentration factor

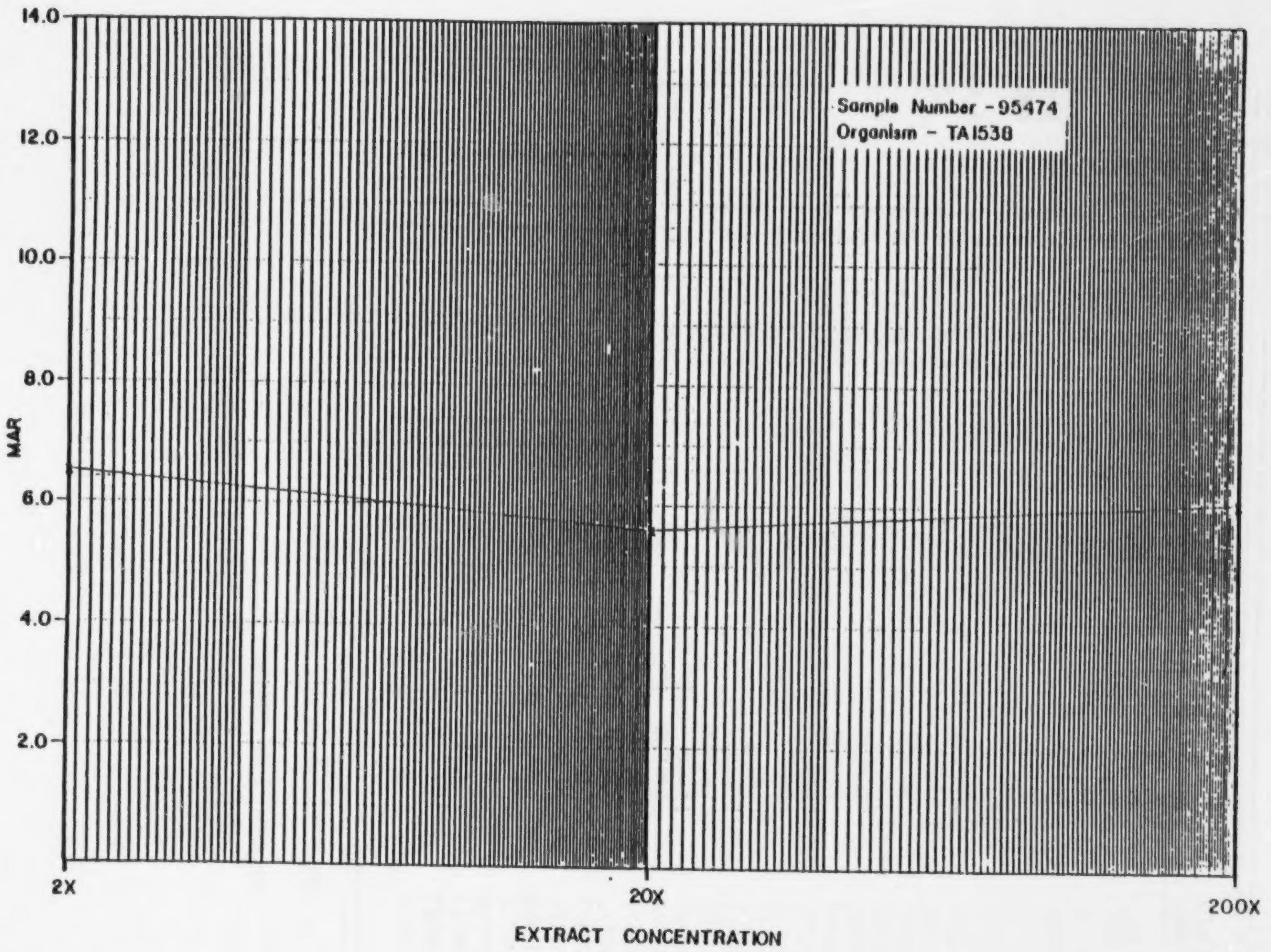


Figure 12. Response Curve for Sample Number 95474.

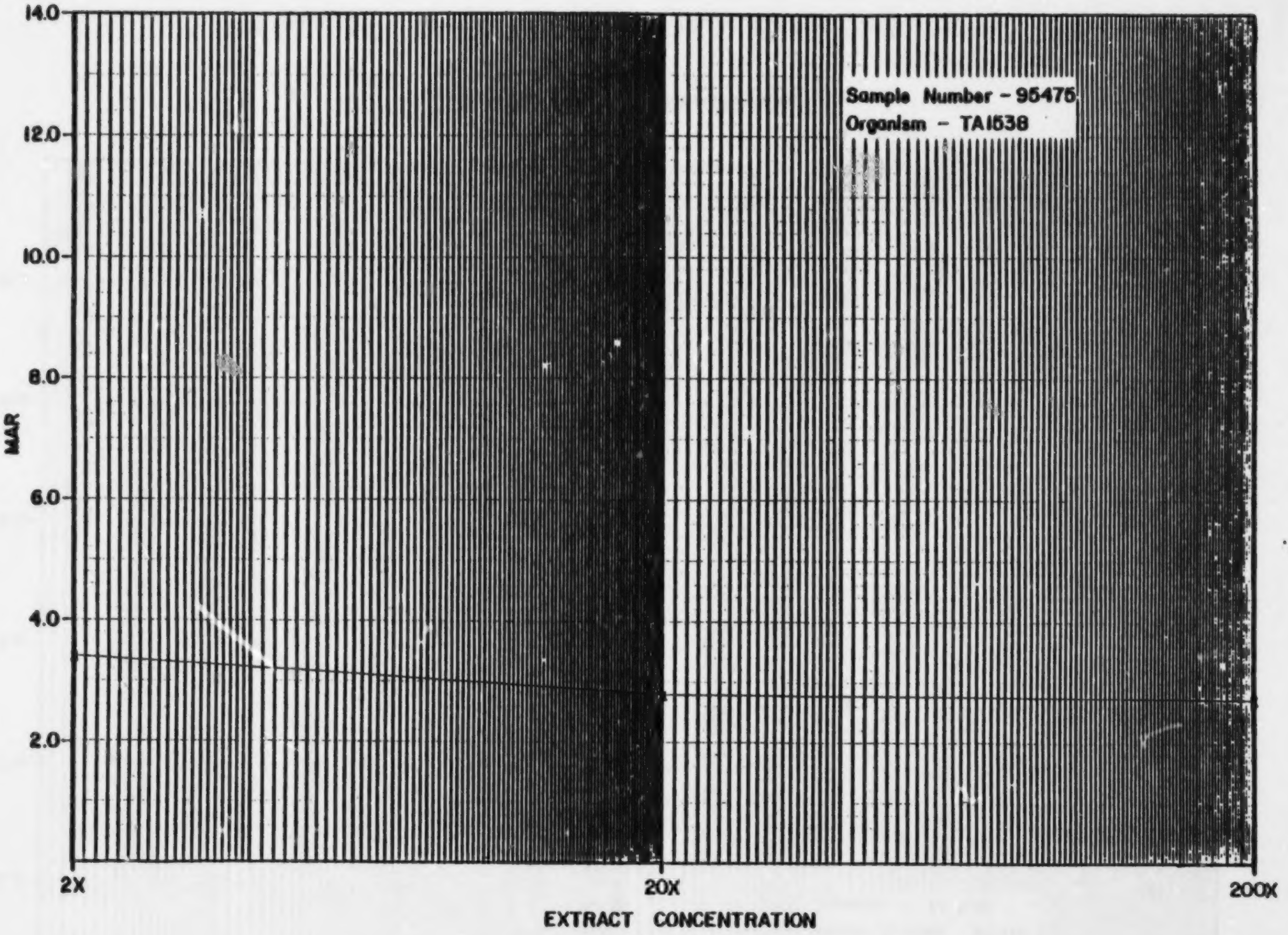


Figure 13. Response Curve for Sample Number 95475.

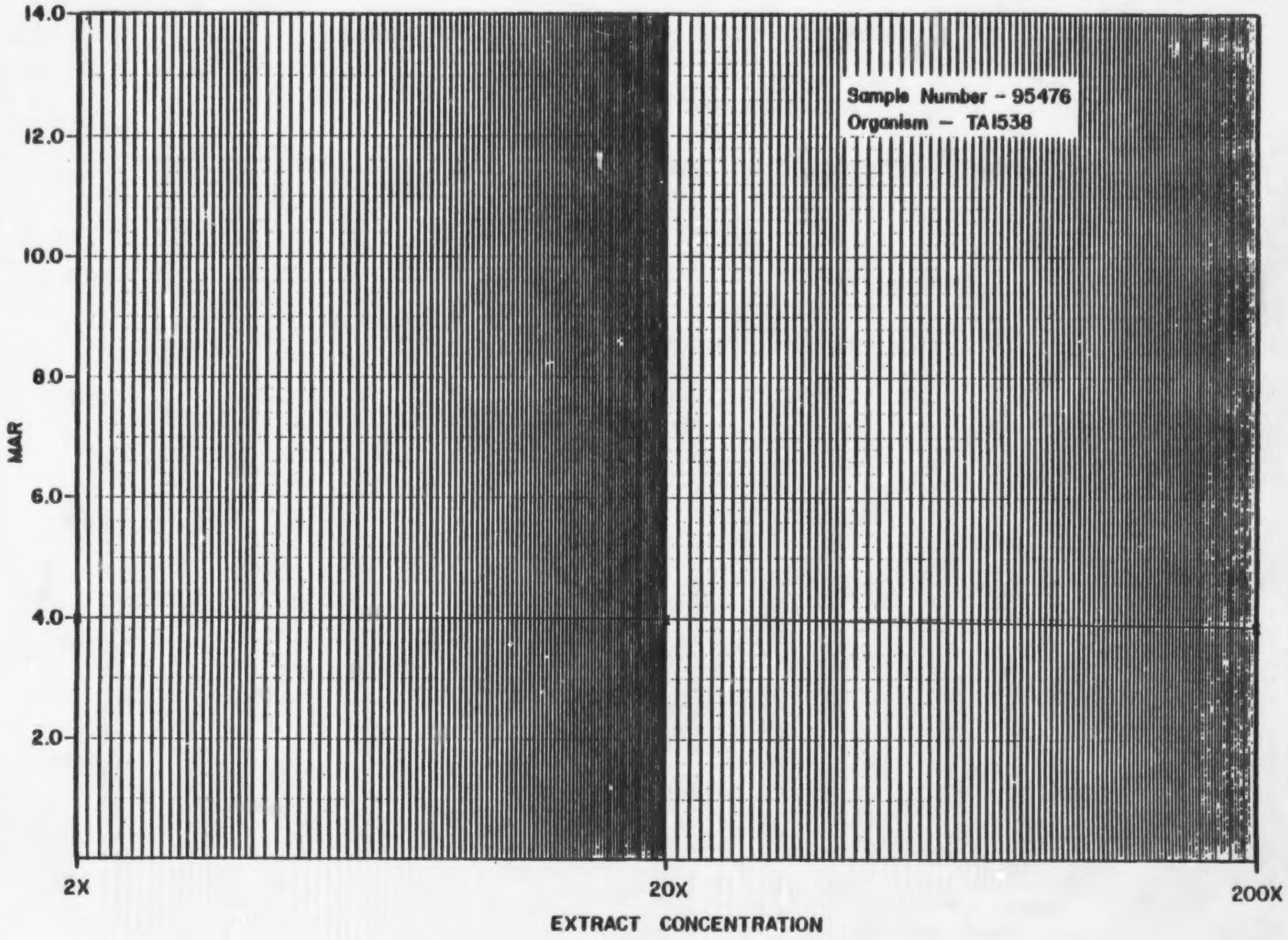


Figure 14. Response Curve for Sample Number 95476.

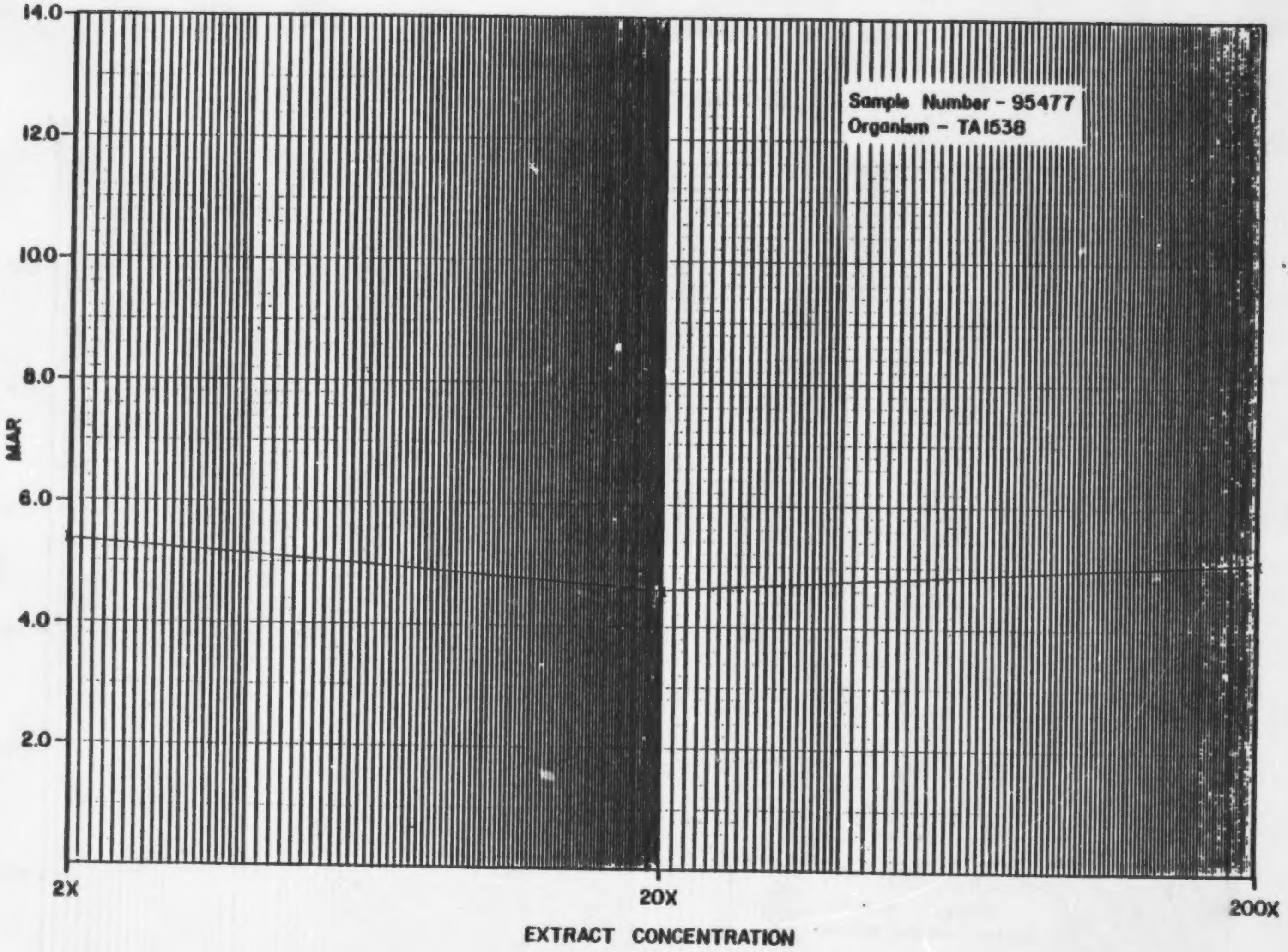


Figure 15. Response Curve for Sample Number 95447.

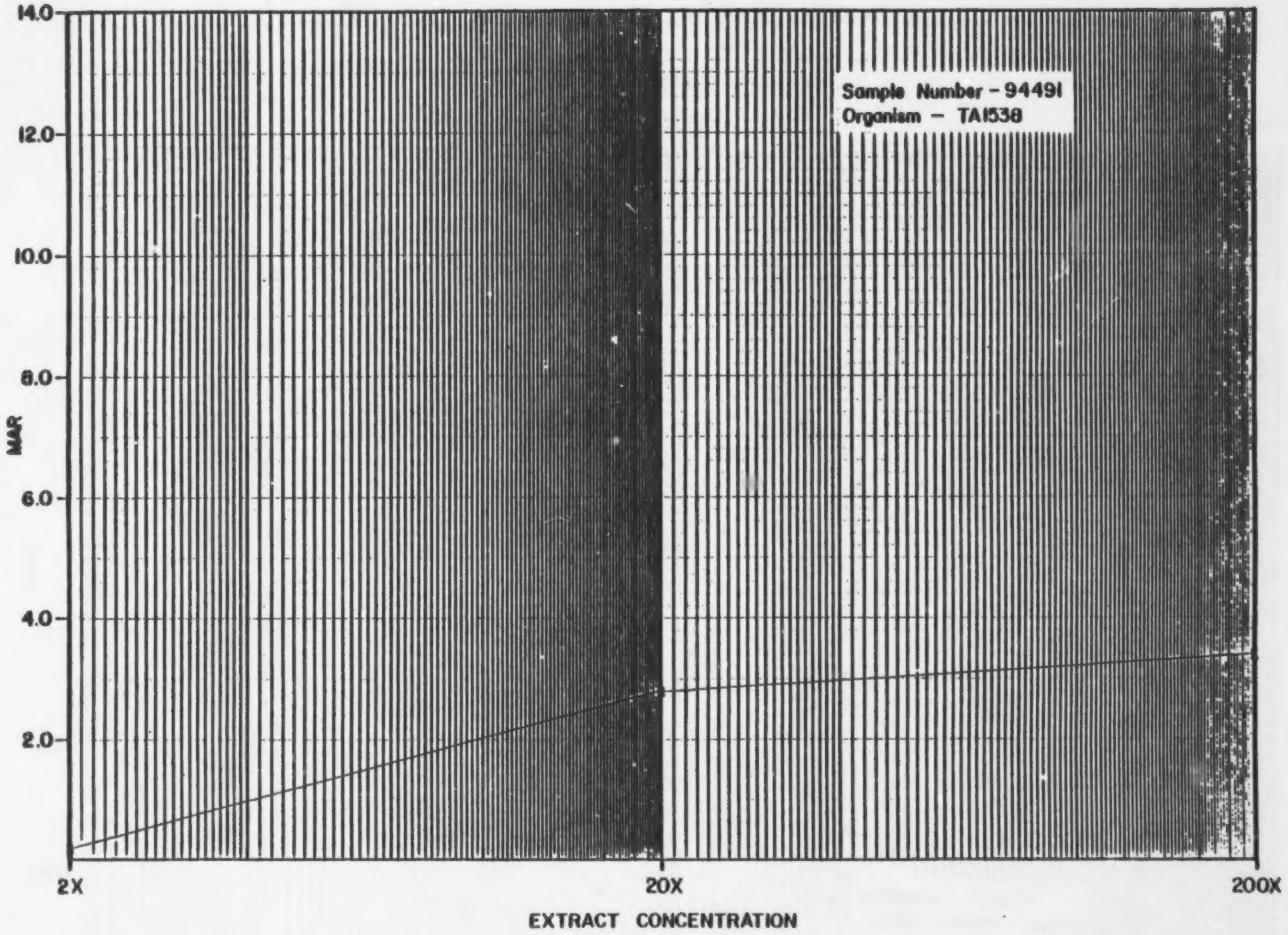


Figure 16. Response Curve for Sample Number 94491.

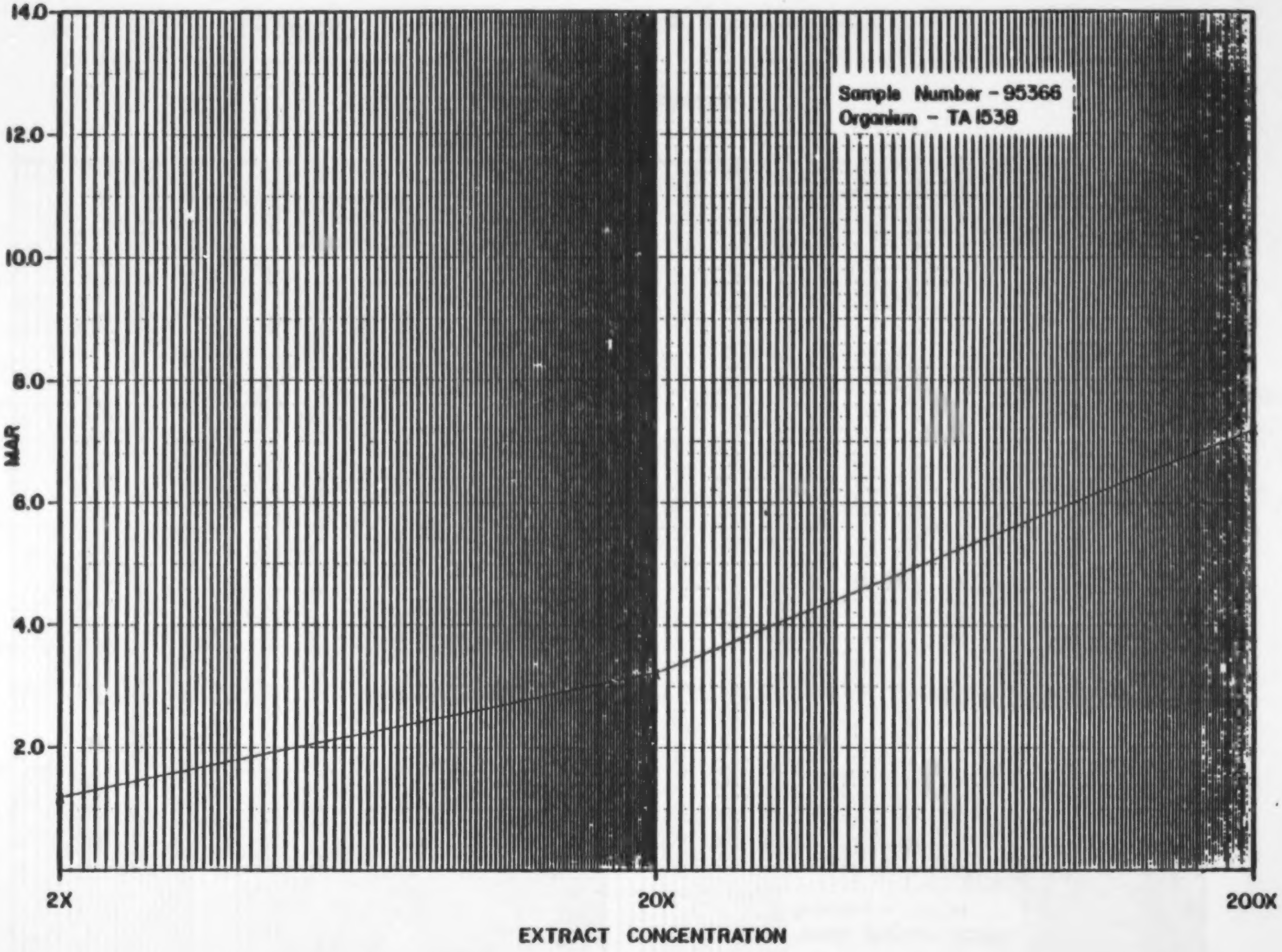


Figure 17. Response Curve for Sample Number 95336.

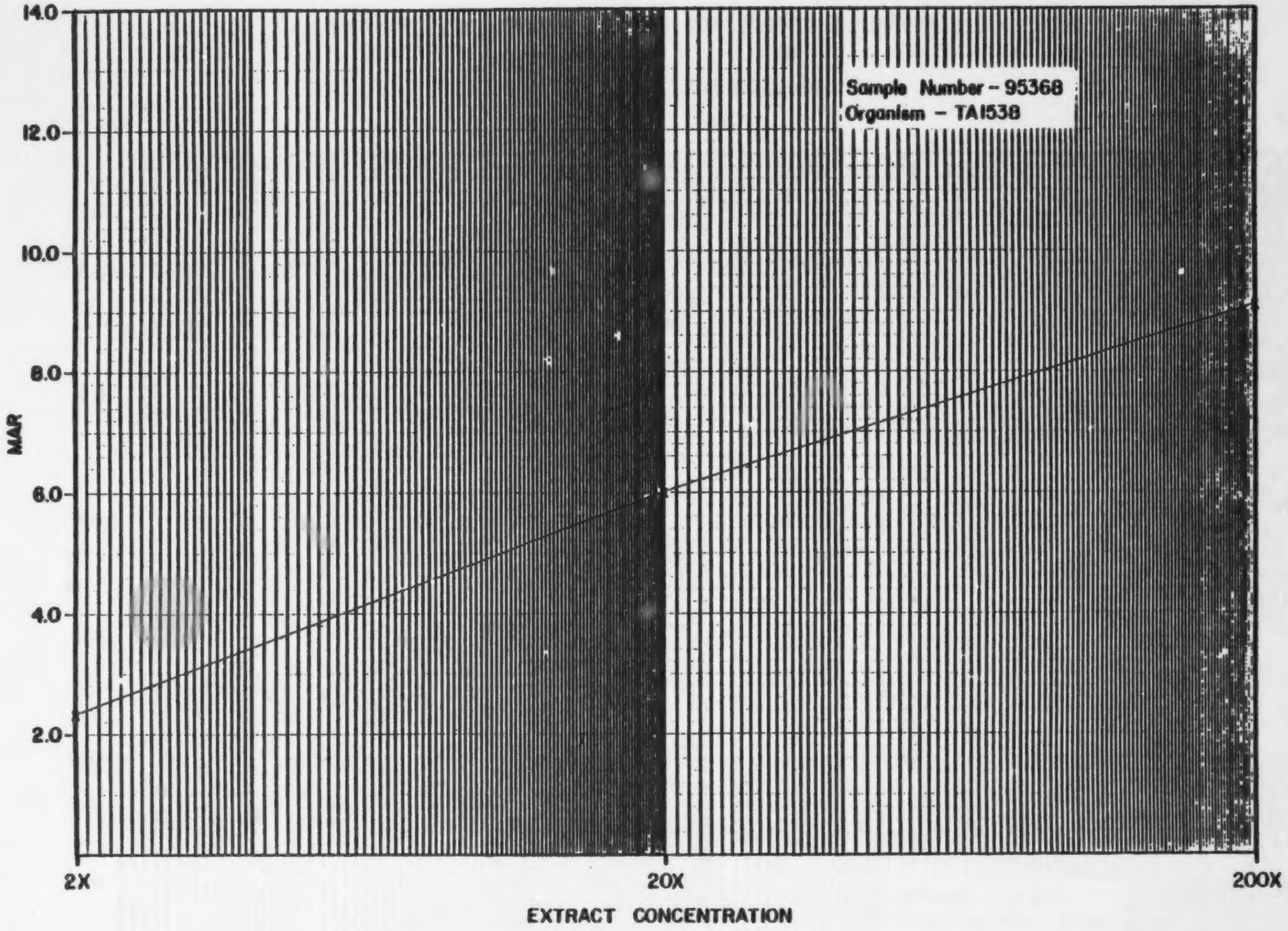


Figure 18. Response Curve for Sample Number 95368.

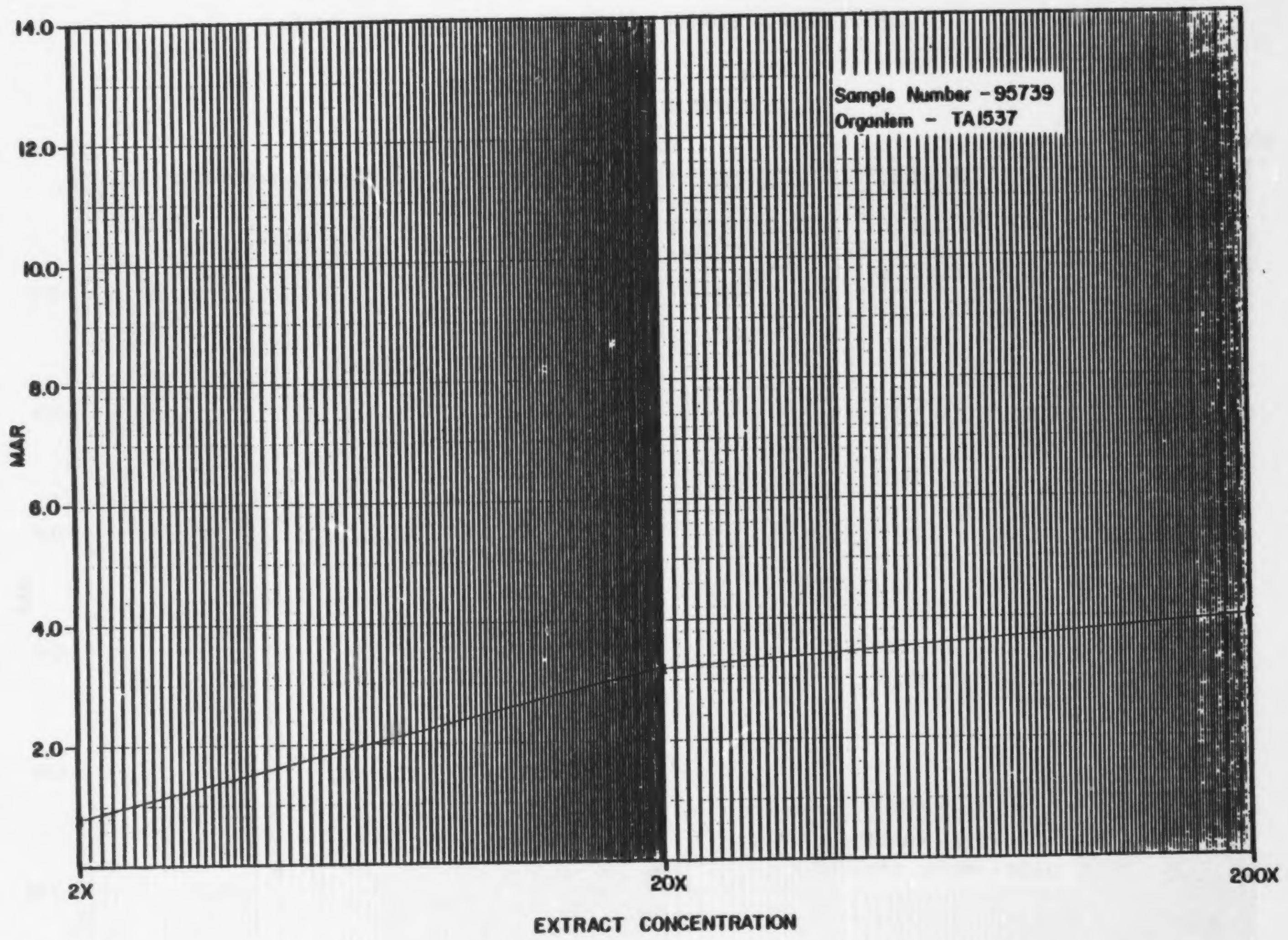


Figure 19. Response Curve for Sample Number 95739.

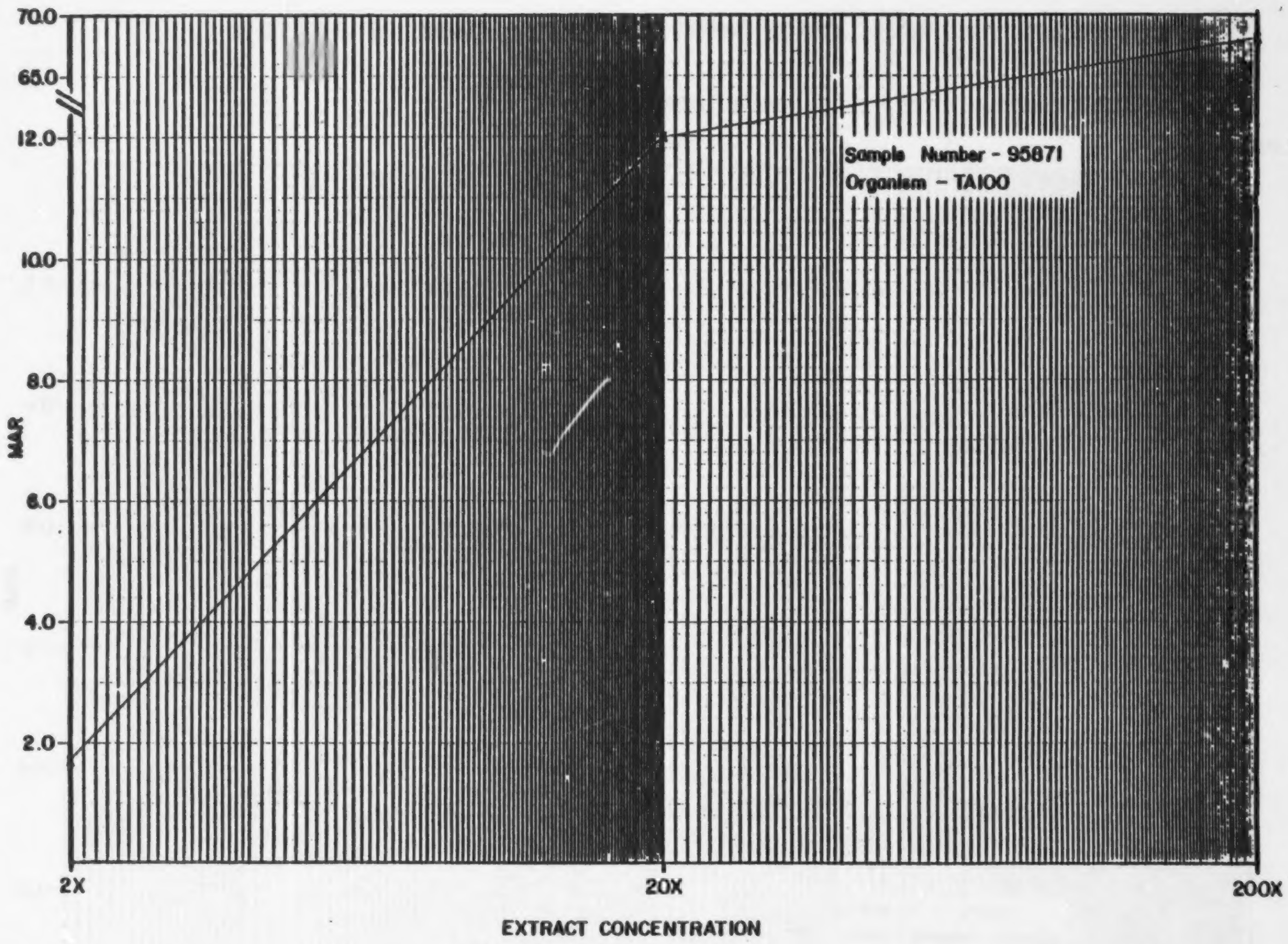


Figure 20. Response Curve for Sample Number 95871.

Response curves are presented in Appendix D for the other ten samples for which a detectable response was observed in at least one sample concentration.

Of the 30 detectable responses (responses from more than one test strain for six of nineteen samples), ten were from site 004 (in three samples), while four were from site 037 (also in three samples). A more interesting observation is that 17 responses of the total of 30 (57%) were from 9 runoff samples (47% of the 19 samples from which detectable responses were obtained. CSO samples produced 7 responses from 5 samples, while dry-weather sanitary samples elicited 4 responses from 3 samples. Therefore, urban stormwater runoff may be a contributor of potentially mutagenic substances to CSO and receiving waters. However, since mutagenic activity is not as evident in the respective CSO samples as it is in the urban runoff, it is probable that the concentrations of mutagenic substances in the runoff are diluted below the detectable limits of this study as the runoff mixes with sanitary waste to form CSO.

Phase II

For the verification phase of the study, an additional eleven samples were collected in Syracuse in late summer of 1981. These samples included two runoff and two CSO samples from both sites 004 and 037, two samples of Metro influent, and a rain sample. One gallon of each sample was extracted, with the concentrated extract brought to a volume of 20 ml with DMSO, resulting in a concentrate of 189.25 times the original sample. Five dosages (500 ul, 400 ul, 300 ul, 200 ul, 100 ul) were applied.

In two instances, the samples did elicit a detectable response. One sample was CSO from site 004, while the other was Metro influent. The CSO sample (OBG No. 28524) had an MAR of 5.2 for tester strain TA 1538 at a dosage of 200 ul; higher dosages resulted in a weaker response. The Metro influent had an MAR of 6.1 for TA1538 at the highest applied dosage of 500 ul; lower dosages did not result in an MAR greater than 2.4.

Two samples produced toxicity: runoff sample site 004 (OBG No. 28885) with TA100 (no activation), Metro influent (OBG No. 28888) with TA100 (with and without activation).

These four samples were re-analyzed for these particular tester strains with results as presented in Table 17. None of the analyses resulted in an MAR of 2.5 or greater at any applied dosage. Response curves for these four samples are presented in Appendix E.

TABLE 17. PHASE II SAMPLE VERIFICATION - MARs

OBG Sample Number	S-9 Strain	Applied Sample Dosage (ul)			
		Mix	500	400	300
28524	TA1538	-	2.2	1.8	0.9
28885	TA100	-	1.4	1.2	1.3
28888	TA100	-	1.6	1.5	1.4
	TA100	500 ul	1.3	0.8	0.5
29031	TA1538	-	2.4	2.1	1.0

EXTRACTIONS

It should be mentioned that the use of a liquid-liquid extraction and subsequent concentration procedure for all samples in this study, as recommended in the EPA methodology, adds a bias to the results. The test extract actually represents the DCM-extractable fraction of the original sample, a fraction which may contain a large portion of the extractable organic compounds in the original environmental sample, but probably not all organics. Other studies have used similar extraction procedures with concentration by solvent reduction (36,51,61,63); resin extraction with solvent desorption and reduction also is used (10,22,56), a familiar technique in sample clean up for organic chemical analysis. The choice of solvent for desorption and the ability of potentially unknown mutagenic compounds in complex environmental samples to be desorbed from resins are factors which may also bias results in these procedures.

Both resin systems and filter sterilization (59) may preclude analysis of organic mutagens adsorbed by particulates or absorbed to oil globules. That is why a total sample disinfection/extraction/concentration procedure was used for this study in an attempt to retain as many potential organic mutagens as possible within the limits of the DCM procedure. However, many potential inorganic mutagens, including heavy metals, were excluded by this procedure. Other concentration procedures utilized in other studies have included low temperature, low pressure distillation (25) and dialysis (61).

In any system, the choice of solvent may be critical for another reason: some known mutagens have been found to lose considerable activity with time spent in a solvent. It is unknown whether this is due to direct mutagen-solvent interactions or some other cause.

FALSE POSITIVE AND FALSE NEGATIVE RESULTS

It cannot be readily determined whether any results obtained in this study may be false positive or negative responses due to the implicit limitations of the test.

Detailed discussions of factors influencing the Ames test to produce false positive or false negative results are available elsewhere (2,5,38,39,55). Several possible explanations exist for false positive results in the Ames test which have significance for highly complex environmental samples and, therefore, at least deserve mention here:

a. the bacterial tester strains contain enzymes capable of metabolizing the chemicals to be tested to active mutagenic forms, in the same manner that the S-9 rat liver microsomal reaction mixture metabolizes pre-carcinogens to their active form. This unanticipated bacterial activation could generate false positives if these bacterial enzymes are not functional in mammals.

b. the bacterial tester strains contain a mutation that deletes the excision repair system of the bacteria. McCann and Ames (39) have described the result of this as to "quantitatively enhance the sensitivity of the system rather than to qualitatively alter the response".

c. substances which have low mutagenic activity have been found not to be carcinogenic upon further testing in animal systems. This may be due more to the statistical limitations of the animal tests which make detection of low activity difficult than to any limitation in the Ames test. In fact, this sensitivity of the Ames test may make it particularly useful for detecting low mutagenic activity in environmental samples that would otherwise be difficult to detect.

By contrast, the problem of false negatives may be of more concern to this test:

a. toxicity of the test substance may cause bacterial cell death. This toxicity may be specific for any or all of the test strains. Generally, the expression of mutagenesis occurs at levels below that of toxicity. However, the toxicity of the test substance may not be related to excessive DNA damage or mutagenic properties, but rather to other cytotoxic properties (such as those of antibiotics).

b. technical inadequacies in the rat liver microsome activation system may prevent detection of substances which require in vitro metabolic activation. The rat liver system may not contain the cofactors for activation of certain substances in the environmental sample; this has been found to be true of some pure compounds.

c. test substances may undergo deactivation, either by biochemical compounds such as cysteine (58) and L-ascorbate (26), or by the S-9 microsomal fraction (14).

d. although the tester strains have been made more sensitive to the transport of test substances into the cell, there is a molecular size limitation to substances which come into contact with the test organism. Since the test substance must enter the bacterial cell to reach and alter the bacterial DNA, sufficiently large molecules are being excluded from the test. Should these large molecules be mutagenic, they will not be detected.

Any of these factors, or a combination of two or more, may be another reason for very low or negative responses of the samples collected in this study. Within these limitations, several general observations can be made as a result of this study:

a. due to the drainage characteristics of the project area, and the necessity of selecting sample collection sites which would provide safe access with reasonable ease, industrial, commercial or mixed land use characterized most of the sites. This is not unusual since most of the catchment areas in the project area are characterized as such. Difficulties were encountered with sample collection from the one predominantly open space catchment area selected.

b. detectable responses were obtained with one or more of the test strains from nineteen samples. Some of these samples required concentration up to two hundred-fold to achieve this result.

c. of 30 detectable responses in 19 samples, 17 responses (57% were from 9 runoff samples. However, the substances responsible for this response may become diluted or inactivated below the detection threshhold of the test by mixing with sanitary sewage to form combined sewage.

In spite of these observations, it should be recognized that a sufficient data base of Ames test results has not been generated by this study to reach conclusions affecting policy decisions, standards or guidelines regarding the necessity of the advanced treatment of CSO or urban runoff. This study should be considered indicative of the need for more comprehensive surveys of its type if biological effects tests of this type are considered useful in the decision-making process.

SECTION 9

SUMMARY

The only known causes of human cancer besides the natural modification of human genetic factors are radiation and chemicals, either natural (such as aflatoxin) or synthetic. The relationship between mutagenic and carcinogenic activity of substances is not fully understood although sufficient empirical evidence is available to indicate that many mutagens are carcinogens (7,31, 39,46).

The introduction of potential mutagens to the human environment may serve to increase the rate of contact with substances that contribute to cancer incidence in the general population. The primary purpose of this study was the evaluation of mutagens in the sanitary environment, including rain, urban runoff, sanitary wastewater, combined sewer overflows, sewage treatment plant influent and effluent, and receiving waters. The study was limited in scope so that the results, if positive, may be indicative of mutagenic activity from a particular source and its life cycle through the system.

At present, the U.S. Environmental Protection Agency is reviewing the extent to which it may be necessary to provide treatment for combined sewer overflows. The potential presence of a mutagenic component in CSO necessitates a further review of the necessity of some form of advanced waste treatment.

This study, conducted on samples collected primarily in Syracuse, NY in conjunction with present system evaluations and improvements, found clear, strong mutagenic activity in several samples when analyzed by the Ames test; data was interpreted under criteria recommended by the EPA. The MAR (equal to or greater than 2.5) rule for assessment of mutagenecity is conservative, and several samples exceeded this value (2.5) substantially indicating likelihood of dose response. Nineteen samples elicited a detectable response to one or more of the five Salmonella typhimurium test strains, (47%) were of urban runoff in the project area, including 17 of a total of 30 detectable responses (MAR equal to or greater than 2.5) (57%). Five of the samples (26%) were from combined sewer overflows, constituting 7 of the 30 detectable responses (23%). However, results indicate that detectable mutagenic activity may be present, particularly in samples of urban runoff. Since the effects of chronic exposure to low level carcinogens is not well known (the identification of threshhold levels and their significance), the criteria for the interpretation and definition of low level mutagens is still controversial, and the concept of a relationship of mutagenic activity in the Ames test to long term animal carcinogenicity testing still draws positive and negative preponents, a more extensive survey of this type should be considered.

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APPENDIX A

This appendix contains the computer tabulation of all physical and chemical data obtained for the samples collected in this study. Except for pH, values listed are in mg/l. The key to the computer identification format may be found in Table 9.

O'BRIEN & GERE ENGINEERS, INC.   LABORATORY DATA SYSTEM   OCT 8, 1981   10:34

SYRACUSE CSO MUTAGENICITY

CITY	SOURCE	LOCATION	SAMP_DES	DATE	TIME	SAMPLE	TYPE	PH	BOD5	TOC	TSS	CD	CR	PB	CU	O&G
1	1	8	1	07/29/80	900	94490		7.3	10.	72.	140.	<.01	<.01	.06	.06	9.0
1	1	8	1	09/17/80	1715	95364		7.0	80.	80.	96.	<.01	<.01	<.01	.04	9.0
1	1	8	1	10/13/80	1005	95740		6.5	60.	95.	160.	<.01	<.01	<.01	.27	52.
1	1	8	1	10/20/80	1300	95864		6.5	90.	77.	96.	<.01	<.01	<.01	.03	52.0
1	1	8	1	09/02/81	1430	28924		7.2								
1	1	8	1	09/04/81	1545	29034		7.3								
1	1	19	1	07/29/80	1100	94492		7.8	10.	35.	510.	.01	<.01	.51	.09	56.
1	1	19	1	09/17/80	1700	95365		9.1	70.	112.	800.	.02	<.01	.64	.35	80.0
1	1	19	1	10/13/80	955	95742		7.5	20.	55.	90.	<.01	<.01	<.01	.10	55.
1	1	19	1	10/20/80	1245	95866		6.8	90.	70.	170.	<.01	.04	<.01	.04	56.0
1	1	27	1	07/29/80	930	94494		7.3	17.	38.	200.	<.01	<.01	.53	.05	8.
1	1	27	1	09/17/80	1720	95366		7.1	33.	80.	113.	<.01	<.01	<.01	.07	8.0
1	1	27	1	10/13/80	1020	95744		3.7	9.	17.	39.	<.01	<.01	<.01	.06	15.
1	1	27	1	10/20/80	1230	95868		6.6	70.	66.	220.	<.01	.21	<.01	.07	60.0
1	1	37	1	07/29/80	950	94496		7.1	14.	42.	280.	<.01	<.01	.23	.03	100.
1	1	37	1	09/17/80	1730	95367		7.1	160.	22.	81.	<.01	<.01	<.01	.09	<1.0
1	1	37	1	10/13/80	1000	95746		6.4	220.	112.	170.	<.01	<.01	<.01	.25	80.
1	1	37	1	10/20/80	1245	95870		6.3	90.	61.	140.	<.01	<.01	<.01	.03	82.0
1	1	37	1	09/02/81	1500	28806		7.6								
1	1	37	1	09/04/81	1600	29032		6.9								
1	1	43	1	07/29/80	1010	94498		7.2	140.	115.	380.	<.01	1.34	.45	.77	120.
1	1	43	1	09/17/80	1745	95368		7.0	140.	91.	400.	.01	.06	.50	.16	41.0
1	1	43	1	10/13/80	1015	95748		7.0	140.	89.	190.	<.01	.02	<.01	.46	102.
1	1	43	1	10/20/80	1300	95872		7.0	160.	600.	250.	<.01	.02	<.01	.09	34.0
1	1	46	1	07/29/80	1040	94500		7.4	410.	65.	1070.	<.01	<.01	.45	.04	100.
1	2	8	1	07/16/80		94251		6.7	60.	31.	90.	<.01	.11	<.01	.08	280.
1	2	8	2	09/25/80	930	95475		7.3	90.	64.	67.	<.01	<.01	<.01	.06	60.
1	2	8	3	10/16/80	830	95813		7.0	120.	61.	180.	<.01	<.01	<.01	.05	115.6
1	2	5	2	09/25/80	1400	95480		5.3	380.	350.	103.	<.01	.04	<.01	.13	28.
1	2	5	3	10/16/80	920	95814		6.8	190.	85.	33.	<.01	<.01	<.01	.09	131.6
1	2	19	1	07/16/80		94252		7.2	100.	45.	330.	<.01	<.01	<.01	.12	90.
1	2	19	2	09/25/80	1000	95476		8.8	140.	80.	30.	.02	<.01	<.01	.05	40.
1	2	19	3	10/16/80	1110	95815		6.8	170.	95.	62.	<.01	<.01	<.01	.06	110.4
1	2	27	1	07/16/80		94253		7.4	130.	9.	<10.	<.01	<.01	<.01	.09	30.
1	2	27	2	09/25/80		95477		7.7	37.	12.	10.	<.01	<.01	<.01	.03	82.
1	2	27	3	10/16/80	1045	95816		7.6	7.	9.	12.	<.01	<.01	<.01	.01	19.6
1	2	37	1	07/16/80		94254		7.0	27.	13.	100.	<.01	<.01	.15	.06	390.
1	2	37	2	09/25/80	830	95478		6.1	470.	330.	800.	<.01	<.01	<.01	.09	74.
1	2	37	3	10/16/80	900	95817			470.	92.	119.	<.01	<.01	<.01	.05	400.8
1	2	43	1	07/16/80		94255		6.0	150.	35.	210.	<.01	.07	<.01	.06	100.
1	2	43	2	09/25/80	850	95479		7.4	100.	62.	170.	<.01	.02	<.01	.09	10.
1	2	43	3	10/16/80	1030	95818			160.	76.	250.	<.01	.05	<.01	.13	100.0
1	2	46	1	07/16/80		94256		6.9	50.	22.	70.	<.01	<.01	<.01	.04	100.
1	3	8	1	07/29/80	900	94491		7.2	15.	56.	400.	.01	.06	.07	.04	130.
1	3	8	1	10/13/80	1005	95739		6.8	30.	107.	500.	<.01	.02	2.06	1.26	40.
1	3	8	1	10/20/80	1300	95865		6.9	60.	76.	210.	<.01	<.01	.55	.03	47.0
1	3	8	1	09/02/81	1430	28805		8.1								
1	3	8	1	09/04/81	1600	29035		7.4								
1	3	19	1	07/29/80	1100	94493		8.3	10.	60.	600.	.01	.03	.30	.09	90.
1	3	19	1	10/13/80	955	95741		6.1	20.	104.	460.	<.01	.02	.99	.28	86.
1	3	19	1	10/20/80	1245	95867		7.5	10.	119.	1190.	<.01	.04	.94	.12	68.0
1	3	27	1	07/29/80	930	94495		8.9	8.	26.	140.	<.01	<.01	.30	.02	56.

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O'BRIEN & GERE ENGINEERS, INC.        LABORATORY DATA SYSTEM            OCT 8, 1981            10134

                                          SYRACUSE CSO MUTAGENICITY

CITY SOURCE LOCATION SAMP DES   DATE   TIME SAMPLE TYPE   PH  BOD5   TOC   TSS   CD   CR   PB   CU    O&G
---- ------ -------- -------- -------- ---- ------ ---- ---- ----- ----- ----- ---- ---- ---- ---- ------
   1      3       27        1 10/13/80 1020  95743       6.2    9.   74.  190. <.01  .02  .85  .21    60.
   1      3       27        1 10/20/80 1230  95869       7.3   24.   56.  310. <.01 <.01  .63  .08   50.0
   1      3       37        1 07/29/80  950  94497       8.0   10.   25.  710. <.01 <.01 <.01  .04   170.
   1      3       37        1 10/13/80 1000  95745       6.8   60.  159. 1050. <.01  .02 1.56  .30   126.
   1      3       37        1 10/20/80 1245  95871       7.0   21.   76.  230. <.01 <.01  .94  .07   11.0
   1      3       37        1 09/02/81 1500  28887       9.0
   1      3       37        1 09/04/81 1000  29033       7.8
   1      3       43        1 07/29/80 1010  94499       7.9   50.   82.  900. <.01 <.01  .45  .02   280.
   1      3       43        1 10/13/80 1015  95747       7.0   40.   91.  780. <.01 <.01  .71  .23    32.
   1      3       43        1 10/20/80 1300  95873       7.4   30.  109.  740. <.01  .04 1.73  .10   12.0
   1      3       46        1 07/29/80 1040  94501       7.3   20.   69.  760. <.01 <.01  .53  .03    90.

   1      4        1        1 11/05/80 1000  96321       7.0    7.  290.   50. <.01  .19 <.01  .08    <1.
   1      4        1        1 09/02/81 1530  28888       7.4
   1      4        1        1 09/04/81 1615  29031       7.4
   1      4        1        2 09/25/80 1020  95473       7.3   51.   46.   30. <.01  .07 <.01  .06    48.
   1      4        2        1 11/05/80 1000  96322       7.3   18.   53.   30.  .01  .07 <.01  .03    <1.
   1      4        2        2 09/25/80 1030  95474       7.5   80.   84.   10. <.01  .07 <.01  .02    35.

   1      5        1        9 10/16/80 1430  95822              2.   12.   90.  .01  .02 <.01  .04   64.4
   1      5        1        9 10/22/80       95932       7.5    3.   28.   20.  .01 <.01 <.01  .02   38.0
   1      5        2        9 10/16/80 1430  95823              6.   12.   95.  .02 <.01 <.01  .01   18.4
   1      5        2        9 10/22/80       95933       7.2    3.   10.   23.  .01 <.01 <.01  .02   18.0

   1      7                   07/29/80  930  94482       4.7    2.    2.   <1. <.01 <.01 <.01 <.01    90.
   1      7                   09/02/80 1025  95097       4.3    5.   <1.    4. <.01 <.01 <.01 <.01    30.
   1      7                   09/17/80 1000  95369       3.8         10.       <.01 <.01 <.01  .12
   1      7                   10/13/80 1100  95749       5.5
   1      7                   09/02/81 1545  28889       6.2


   2      1       28        1 08/10/80       94765       6.5   10.   41.   40. <.01 <.01  .21  .03   140.
   2      1       34        1 08/10/80       94766       6.5   10.   26.   70. <.01 <.01 <.01  .02    68.
   2      1       38        1 08/15/80 1220  94847       6.8   60.   38.  120. <.01 <.01 <.01  .07    16.


   4      1        7        1 08/05/80 1400  94660       7.1    9.   27.    8. <.01 <.01 <.01  .01   120.
   4      1        7        1 08/05/80 1400  94661       7.1    4.   22.    9. <.01 <.01 <.01  .01    60.

   4      3        7        1 08/05/80 1400  94662       7.3   50.   27.  300. <.01 <.01  .43  .05    60.
   4      3        7        1 08/05/80 1400  94663       7.3   15.   31.  280. <.01 <.01  .43  .05    80.


  88     88       88       88 07/30/80       94508
  88     88       88       88 08/06/80       94636
  88     88       88       88 08/14/80       94780
  88     88       88       88 08/14/80       94781
  88     88       88       88 09/16/80       95325
  88     88       88       88 09/16/80       95326
  88     88       88       88 09/16/80       95327
  88     88       88       88 09/16/80       95328
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O'BRIEN & GERE ENGINEERS, INC.          LABORATORY DATA SYSTEM          OCT 8, 1981          10:34

                                              SYRACUSE CSO MUTAGENICITY

CITY SOURCE LOCATION SAMP_DES  DATE     TIME SAMPLE TYPE PH   BOD5  TOC   TSS   CD   CR   PB   CU   O&G
---- ------ -------- -------- -------- ---- ------ ---- ---- ----- ----- ----- ---- ---- ---- ---- ------
 88     88       88        88 09/16/80      95329
 88     88       88        88 09/16/80      95330
 88     88       88        88 09/16/80      95331
 88     88       88        88 09/16/80      95332
 88     88       88        88 09/16/80      95333
 88     88       88        88 09/26/80      95496
 88     88       88        88 09/26/80      95497
 88     88       88        88 10/24/80      95940
 88     88       88        88 10/24/80      95941
 88     88       88        88 11/12/80      96255
 88     88       88        88 11/12/80      96256
 88     88       88        88 11/17/80      96391
 88     88       88        88 11/17/80      96392
 88     88       88        88 11/19/80      96450
 88     88       88        88 11/19/80      96451
 88     88       88        88 09/17/81      38603
 88     88       88        88 09/17/81      38604

 99     99       99        99 07/30/80      94507
 99     99       99        99 08/06/80      94635
 99     99       99        99 08/14/80      94782
 99     99       99        99 09/26/80      95498
 99     99       99        99 10/24/80      95942
 99     99       99        99 11/12/80      96257
 99     99       99        99 11/17/80      96393
 99     99       99        99 11/19/80      96452
 99     99       99        99 09/17/81      38605
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APPENDIX B

The following table contains MAR values for all samples. These values were obtained at a dosage of 100 ul of sample at a concentration of 200X. Values at less concentrated applications are presented in Table 16 for those samples with an MAR equal to or greater than 2.5 for one or more strains.

OBG Sample Number	MAR Without S9	MAR With S9	MAR Without S9	MAR With S9	MAR Without S9	MAR With S9	MAR Without S9	MAR With S9	MAR Without S9	MAR With S9
	TA98		TA100		TA1535		TA1537		TA1538	
94490	1.0	-0.2	0.6	-0.2	0.4	0.2	0.4	0.8	0.2	0
94491	1.2	3.2	1.2	0.2	1.6	0.2	4.1	2.6	3.4	2.5
94492	1.2	2.9	2.8	0.1	1.4	0.2	0.5	1.1	2.2	1.6
94493	1.9	0.5	1.3	0.3	2.6	-0.2	1.1	0.3	1.1	0.5
94494	2.0	2.1	1.0	0	0.8	0.1	0.7	0.9	0.9	2.3
94495	1.6	0.9	1.3	0	0.2	0.2	0.4	-0.2	1.6	1.2
94496	1.0	0.2	0.5	-0.1	0.1	-0.3	0.8	-0.7	0.9	-0.1
94497	2.7	0.8	1.0	0.1	0.7	0	0.6	0	1.6	0.1
94498	0.9	1.0	-1.2	-0.2	-0.2	-0.5	0.1	0.1	-0.3	-0.1
94499	1.6	1.6	0.2	0.2	-0.4	-0.2	-1.3	0.1	0	0
94255	-0.5	-0.3	0.1	0.1	0.5	-0.3	-0.3	0.2	0.4	0.5
94256	0	0	-0.3	-0.1	0.3	0.3	-0.9	-0.2	b	b
94482	0.1	0	0	0	0.1	0.1	-0.2	-0.1	0.6	0.6
94660	0	-0.2	0.1	-0.1	0.3	0.3	0.2	0.9	1.3	1.3
94500	1.0	0.8	-0.1	0.9	0.2	0.2	0.5	1.9	-0.3	1.9
94501	1.7	1.0	0.2	0.4	0.7	0	2.4	1.8	0.7	1.2
94661	0.2	0.7	-0.5	0.2	-0.4	-0.5	-0.5	0.6	-1.0	0.7
94662	0.2	1.0	-0.1	0.8	-0.3	0.4	0.2	1.4	4.5	0.7
94663	0.8	1.0	0.2	0.3	0.5	0.6	1.0	2.3	3.8	1.3
94765	0.7	1.5	0	0.4	0.2	0.7	1.8	1.3	4.4	0.5
94766	0	0.6	16.2	b	b	b	4.2	b	-0.7	b

OBG Sample Number	MAR TA98		MAR TA100		MAR TA1535		MAR TA1537		MAR TA1538	
	Without S9	With S9	Without S9	With S9	Without S9	With S9	Without S9	With S9	Without S9	With S9
94847	-0.7	0.1	a	-0.4	-1.2	0.1	0.5	0	-0.5	0.6
95364	0	-0.4	0	-0.1	-0.4	-0.7	-0.2	-0.4	0.2	1.1
95365	0.1	0.4	0.2	0.2	-0.3	-0.1	0.2	0	0.8	1.9
95366	0	0.8	-0.4	-0.3	-0.5	-0.7	a	0.6	0	7.2
95367	0.2	0.7	a	-0.2	0.2	0.2	0.5	-0.3	0	2.2
95368	0.3	0.3	a	0.1	-0.3	0.2	a	0.3	0	9.1
95369	0.3	0.2	0.5	0	-0.2	-0.3	0.8	0.3	0	2.6
95473	0	-0.1	a	-0.3	0.2	-0.1	a	0.3	a	1.5
95096	-0.5	1.6	b	b	-0.4	-0.3	b	-1.2	b	-1.1
95496	0.3	-0.4	2.3	2.2	0.2	-0.2	1.2	-0.8	-0.3	0.2
95497	-0.2	0.7	0.4	0	0.6	-0.5	1.0	0.2	-0.8	0.1
95739	3.0	2.6	0.2	0.4	0.5	0.5	4.0	4.8	1.3	2.0
95474	0.5	0.4	0	0.5	-0.2	0.4	0.5	-0.3	-0.8	6.1
95475	-0.1	1.0	0	0.3	-0.2	1.0	-0.8	-0.4	a	2.7
95476	0.7	0.7	0	0	-0.2	0.1	1.5	-0.1	a	3.9
95477	0	0.8	-0.1	0.1	0.2	0.2	1.5	0	-0.3	5.1
95480	c	c	a	0	0.2	0	0.5	-0.7	a	-3.5
95864	c	c	-0.3	0	-0.5	-0.3	0	-0.3	1.0	-2.9
95478	c	c	-0.2	0	b	b	-0.5	b	a	-3.2
95940	c	c	-0.1	0.2	-0.6	0.2	-0.5	-0.3	0.3	-2.1
95941	c	c	-0.5	-0.1	0.3	-0.8	0.2	0.2	0.3	-2.3
95942	c	c	-0.2	0.1	-0.9	-0.5	-0.1	-0.4	0.3	-2.4
94479	c	c	a	0.1	a	-0.1	a	-0.2	a	-3.0
95741	-0.3	-0.6	0.1	-0.1	1.5	0.8	0.2	-0.2	1.3	2.0
95742	a	-0.7	b	b	b	b	b	b	b	b
95740	-1.0	-0.7	-0.1	0.1	b	-0.8	b	-1.8	b	b
95744	-0.9	a	b	-0.2	b	b	0.8	-1.4	b	b
95743	-1.2	-2.3	0.2	a	0	-0.8	-0.5	-1.3	-0.2	-1.0
95745	-0.8	-1.0	a	0	b	b	1.5	-0.8	b	2.9

OBG Sample Number	MAR Without S9 TA98	MAR With S9 TA98	MAR Without S9 TA100	MAR With S9 TA100	MAR Without S9 TA1535	MAR With S9 TA1535	MAR Without S9 TA1537	MAR With S9 TA1537	MAR Without S9 TA1538	MAR With S9 TA1538
96253	-0.8	-0.6	-0.3	0.3	a	0.6	0.2	0	2.2	-0.3
95746	-1.2	-0.8	a	0.1	b	0	a	b	b	b
95748	-1.2	-1.0	-0.5	0.4	b	-0.5	-0.5	-0.6	b	-0.3
95477	-1.0	-0.6	b	11.2	b	-0.5	a	0.4	b	a
95815	-1.0	a	a	0.2	a	a	-0.8	-1.0	a	a
95816	-0.6	-0.4	-0.1	0.3	b	a	-1.2	-0.4	-0.2	
95813	b	-0.3	b	0.2	-0.1	b	2.0	-0.9	b	0.1
95814	b	b	-0.2	0.4	b	0.1	-0.3	-1.1	b	-0.2
95871	-0.3	-0.1	68.	29	b	-0.3	-1.2	-0.3	-0.3	b
95870	-0.8	-0.3	b	b	-0.6	0	0	-1.0	0.2	1.2
95872	-0.8	-0.6	b	b	-0.5	-0.6	0.8	b	0	-0.9
95869	-0.3	-0.2	1.0	b	-0.6	-0.9	-0.5	0.3	-0.3	-0.9
95873	-0.4	-0.7	b	0.1	b	b	-0.4	-0.3	0	10.3
95817	1.1	0.3	0.2	0	1.5	0.8	b	-0.6	0.8	-0.2
95818	0.4	0	-0.2	-0.1	1.3	0.2	0.5	-0.6	0.8	0.1
95822	0	0.3	0.3	0.2	0.3	-0.1	-0.8	-0.1	0.8	-0.4
95823	0.1	-0.5	0.5	0	0.3	1.0	0.8	0.4	0.8	0.4
95865	0.9	0.6	0.7	0.5	1.0	0.8	-0.2	0.1	1.3	0.2
95866	0.1	1.0	0	-0.3	-0.4	-0.2	0.5	-0.1	a	0.5
95867	b	b	0	-0.2	0.3	-0.6	-0.8	0.4	-0.4	-0.7
95868	-0.7	0	0	0.2	-0.8	0.1	-0.2	0	a	a

a - toxicity of raw concentrate (200X sample) as applied (100 ul); lesser concentrations did not elicit a mutagenic response.

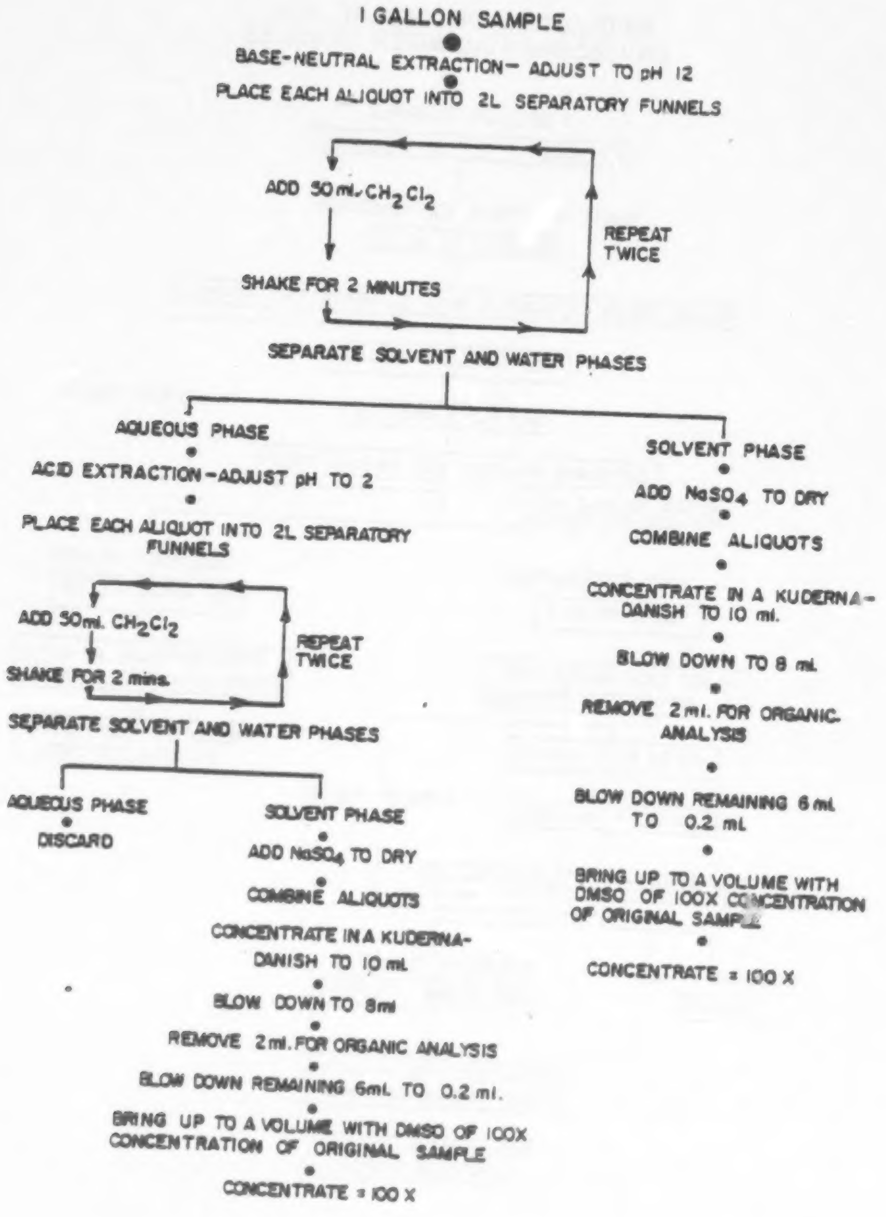
b - sample toxic at all concentrations.

c - laboratory error

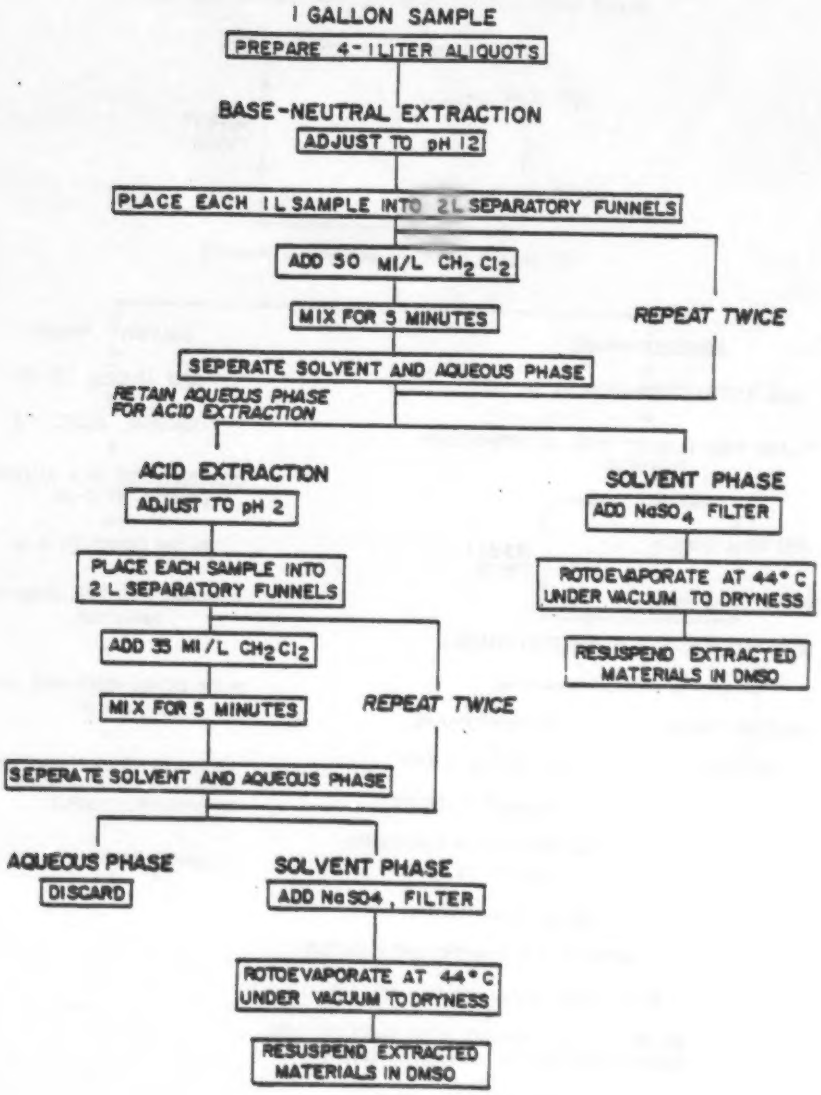
APPENDIX C

The following two flow charts summarize the extraction and test procedures used by the EPA Region II Environmental Services Laboratory (Edison, NJ) for split sample analysis.

AMES TEST EXTRACTION PROCEDURE FOR ENVIRONMENTAL WATER SAMPLES

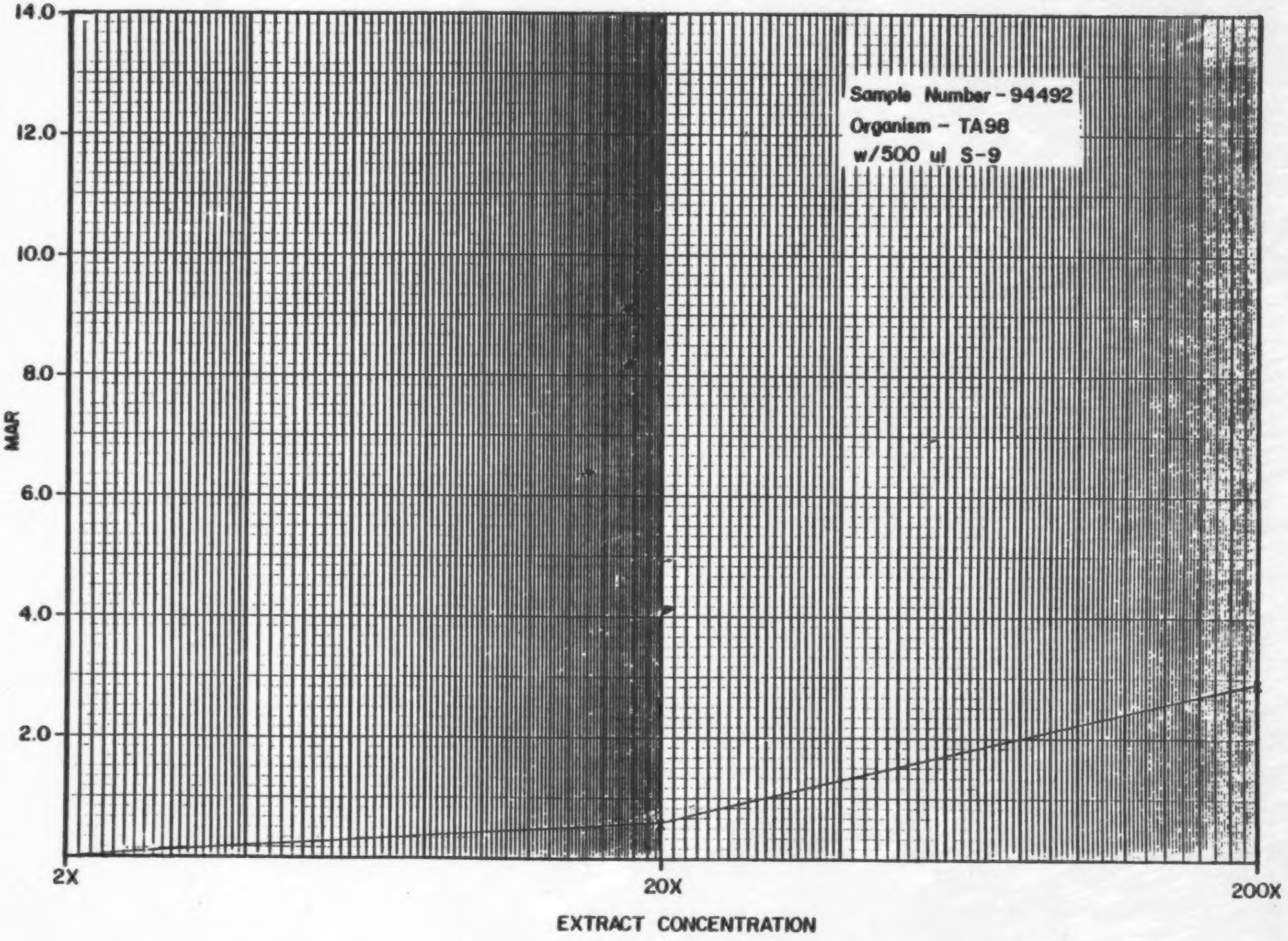

I GALLON SAMPLE
BASE-NEUTRAL EXTRACTION– ADJUST TO pH 12
PLACE EACH ALIQUOT INTO 2L SEPARATORY FUNNELS
ADD 50 ml. CH_2Cl_2
SHAKE FOR 2 MINUTES
REPEAT TWICE
SEPARATE SOLVENT AND WATER PHASES
AQUEOUS PHASE
ACID EXTRACTION–ADJUST pH TO 2
PLACE EACH ALIQUOT INTO 2L SEPARATORY FUNNELS
ADD 50ml. CH_2Cl_2
SHAKE FOR 2 mins.
REPEAT TWICE
SEPARATE SOLVENT AND WATER PHASES
AQUEOUS PHASE
DISCARD
SOLVENT PHASE
ADD $NaSO_4$ TO DRY
COMBINE ALIQUOTS
CONCENTRATE IN A KUDERNA-DANISH TO 10 ml.
BLOW DOWN TO 8ml
REMOVE 2ml. FOR ORGANIC ANALYSIS
BLOW DOWN REMAINING 6ml. TO 0.2 ml.
BRING UP TO A VOLUME WITH DMSO OF 100X CONCENTRATION OF ORIGINAL SAMPLE
CONCENTRATE = 100 X
SOLVENT PHASE
ADD $NaSO_4$ TO DRY
COMBINE ALIQUOTS
CONCENTRATE IN A KUDERNA-DANISH TO 10 ml.
BLOW DOWN TO 8 ml.
REMOVE 2 ml. FOR ORGANIC ANALYSIS
BLOW DOWN REMAINING 6 ml. TO 0.2 ml.
BRING UP TO A VOLUME WITH DMSO OF 100X CONCENTRATION OF ORIGINAL SAMPLE
CONCENTRATE = 100 X

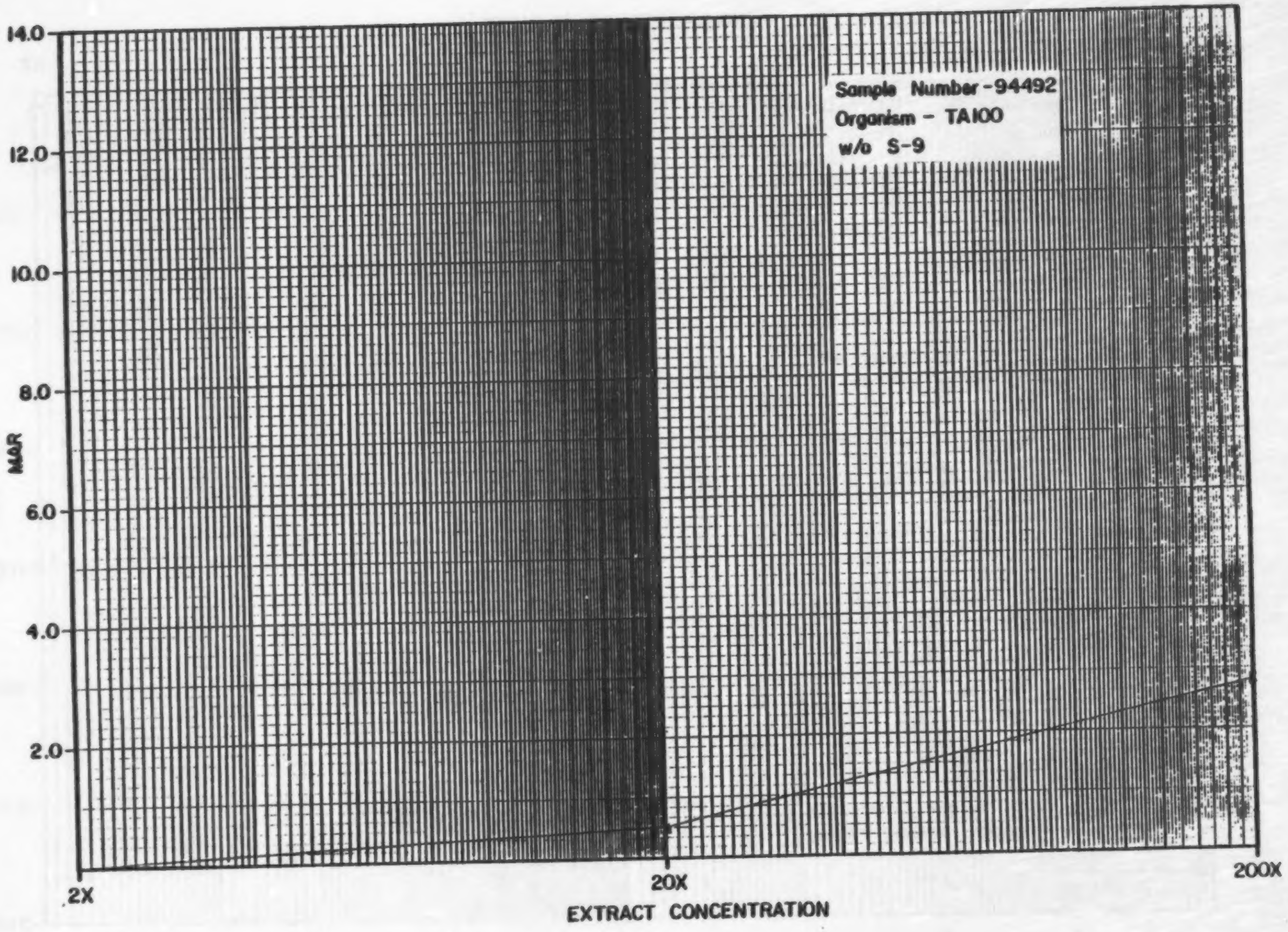
EXTRACTION PROCEDURE FOR ENVIRONMENTAL WATER SAMPLES

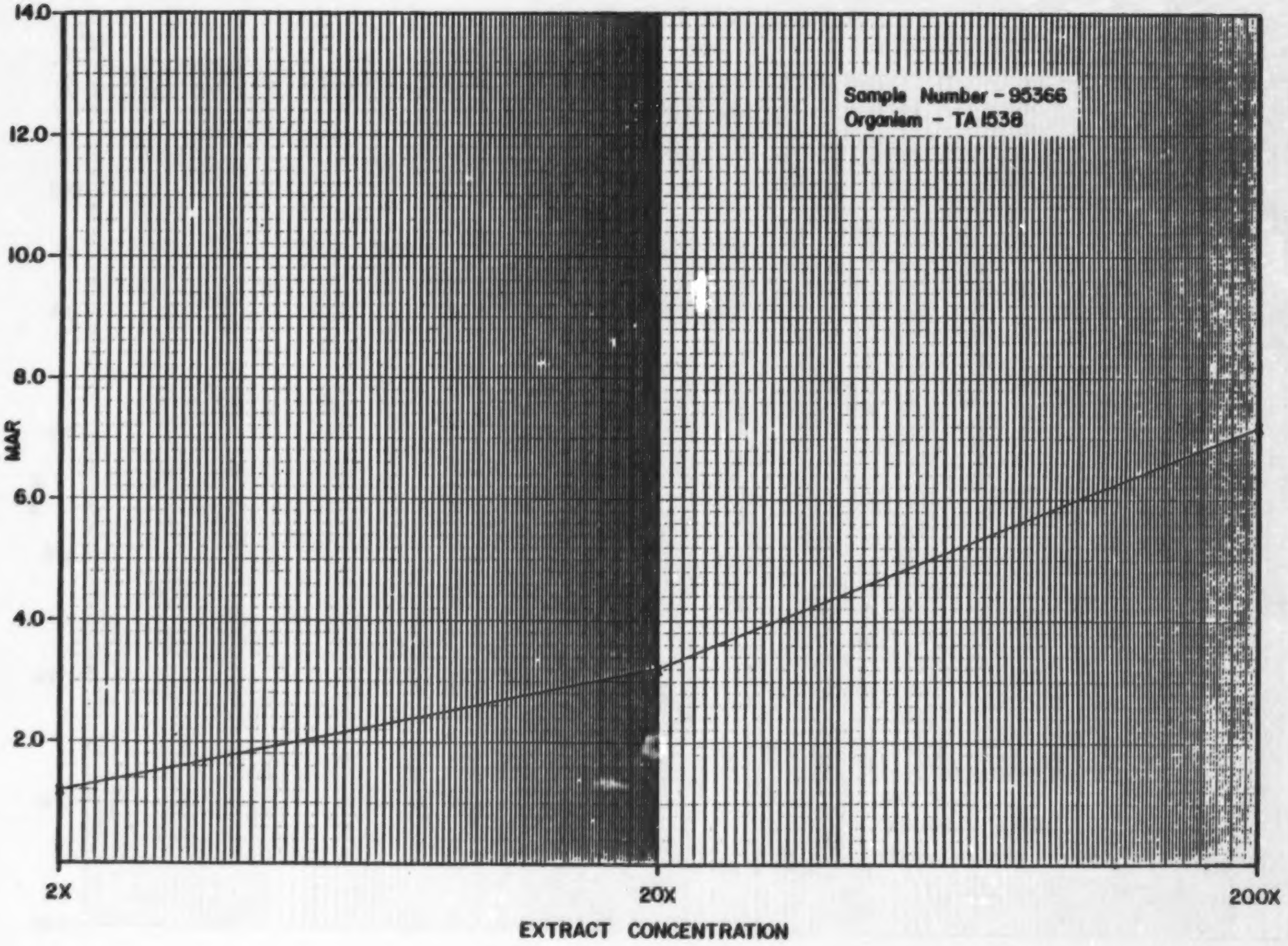

I GALLON SAMPLE
PREPARE 4-1LITER ALIQUOTS
BASE-NEUTRAL EXTRACTION
ADJUST TO pH 12
PLACE EACH 1L SAMPLE INTO 2L SEPARATORY FUNNELS
ADD 50 Ml/L CH2Cl2
MIX FOR 5 MINUTES
REPEAT TWICE
SEPERATE SOLVENT AND AQUEOUS PHASE
RETAIN AQUEOUS PHASE FOR ACID EXTRACTION
ACID EXTRACTION
ADJUST TO pH 2
PLACE EACH SAMPLE INTO 2 L SEPARATORY FUNNELS
ADD 35 Ml/L CH2Cl2
MIX FOR 5 MINUTES
REPEAT TWICE
SEPERATE SOLVENT AND AQUEOUS PHASE
AQUEOUS PHASE
DISCARD
SOLVENT PHASE
ADD NaSO4, FILTER
ROTOEVAPORATE AT 44° C UNDER VACUUM TO DRYNESS
RESUSPEND EXTRACTED MATERIALS IN DMSO
SOLVENT PHASE
ADD NaSO4 FILTER
ROTOEVAPORATE AT 44° C UNDER VACUUM TO DRYNESS
RESUSPEND EXTRACTED MATERIALS IN DMSO

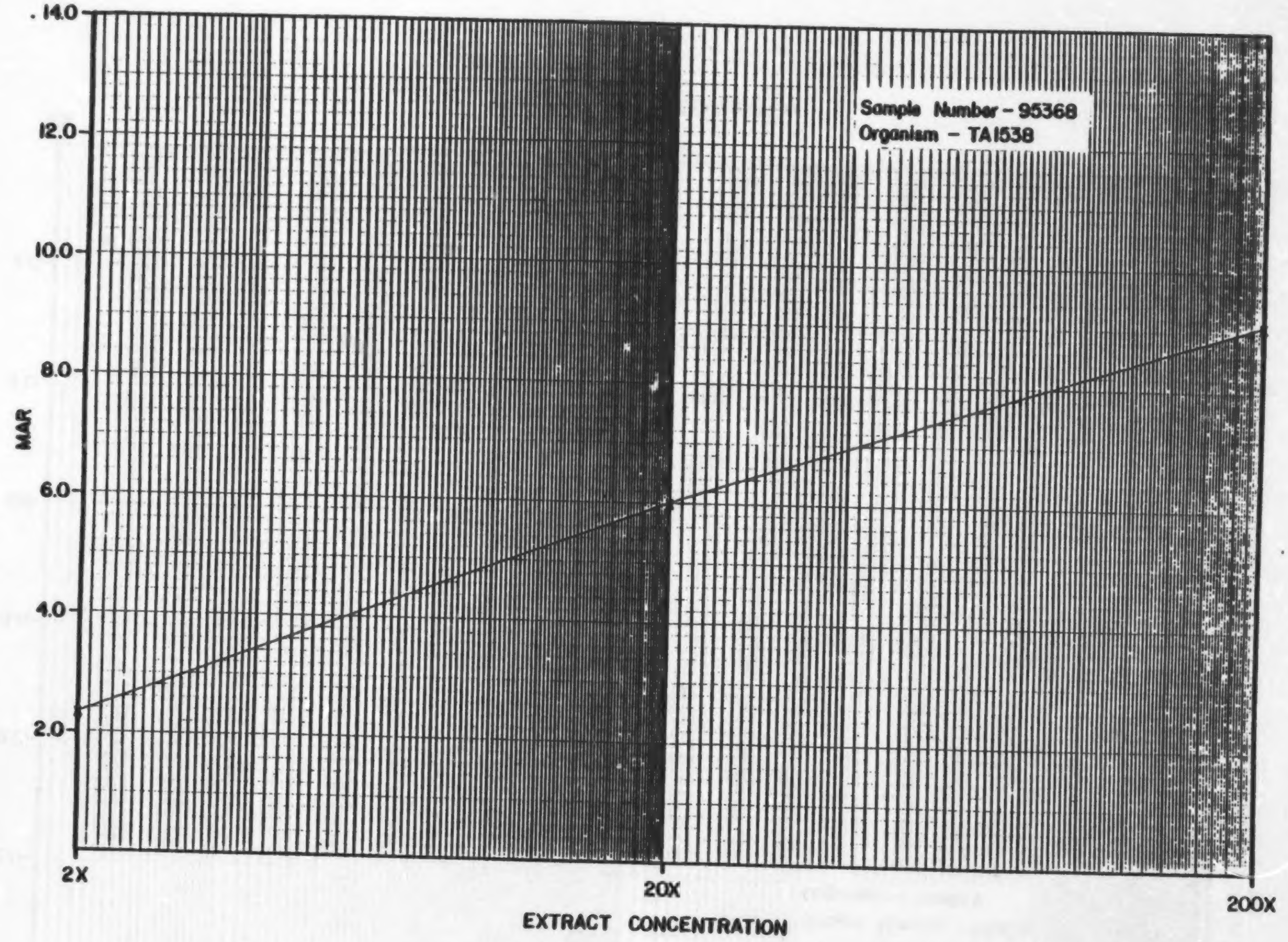
APPENDIX D

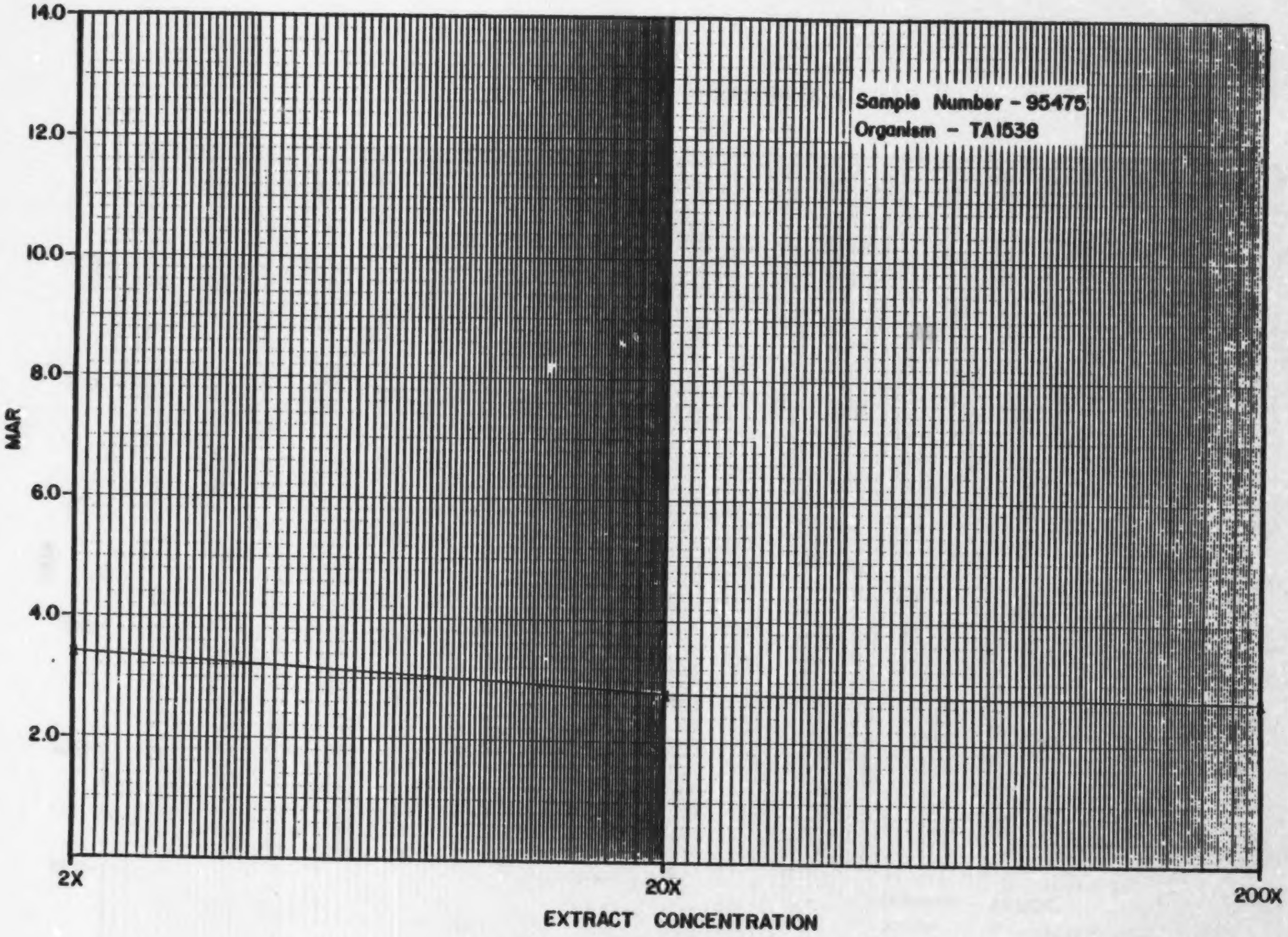
Dose Response Curves for Samples Without Detectable Mutagenicity - Phase I

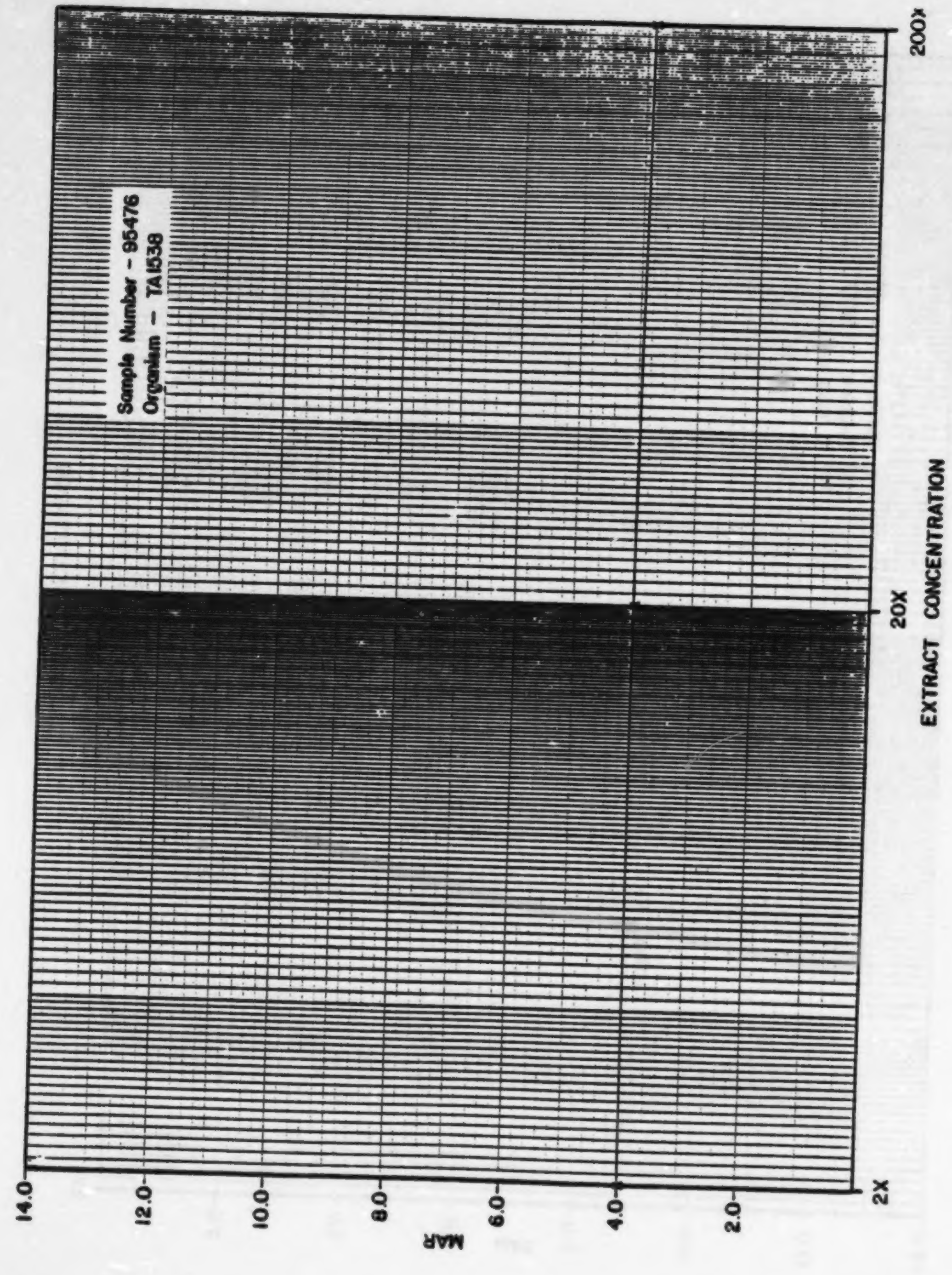

Sample Number - 94492
Organism - TA98
w/500 ul S-9
MAR
14.0
12.0
10.0
8.0
6.0
4.0
2.0
2X
20X
200X
EXTRACT CONCENTRATION

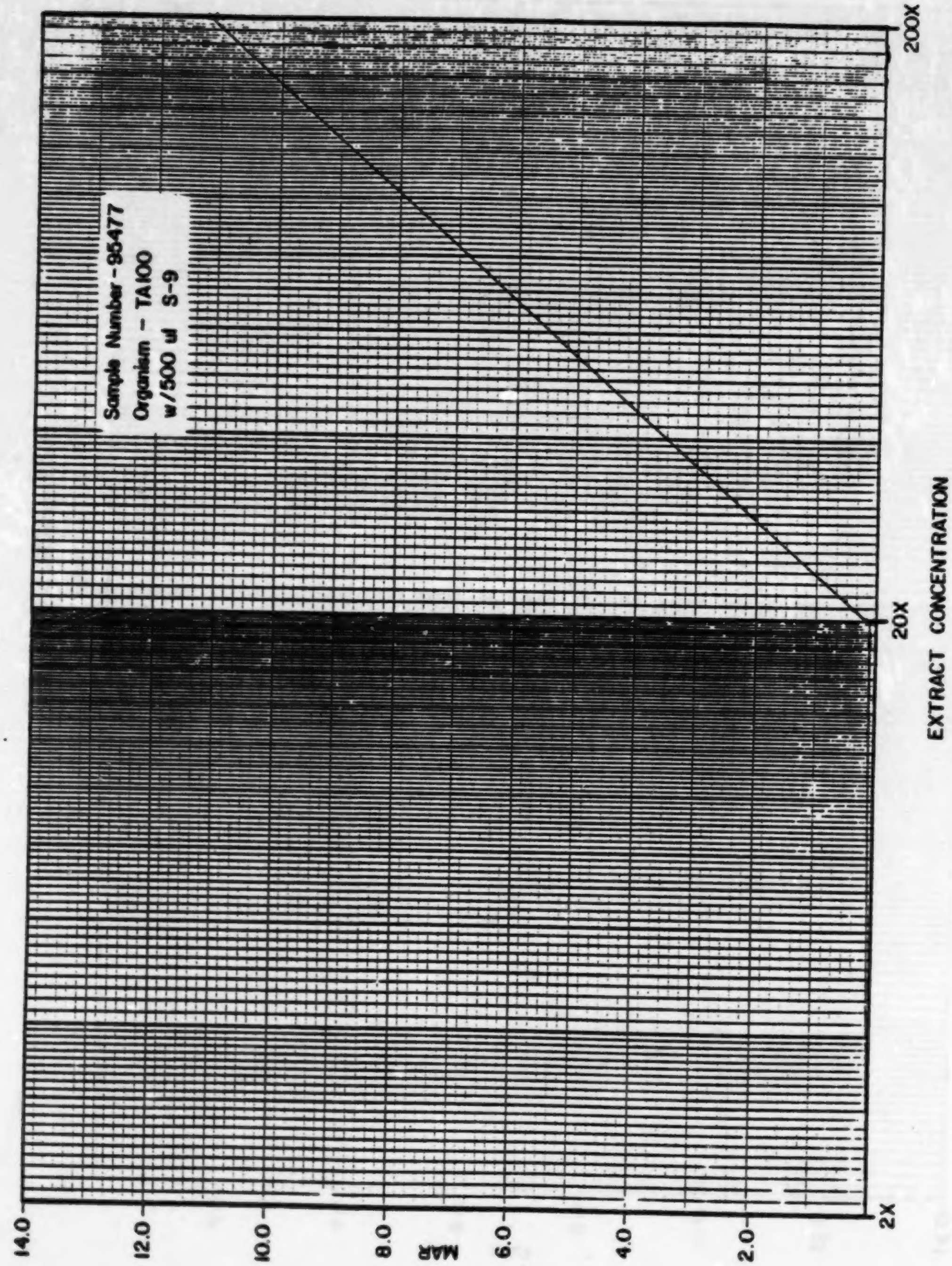

Sample Number -94492
Organism - TA100
w/o S-9
14.0
12.0
10.0
8.0
MAR
6.0
4.0
2.0
2X
20X
200X
EXTRACT CONCENTRATION

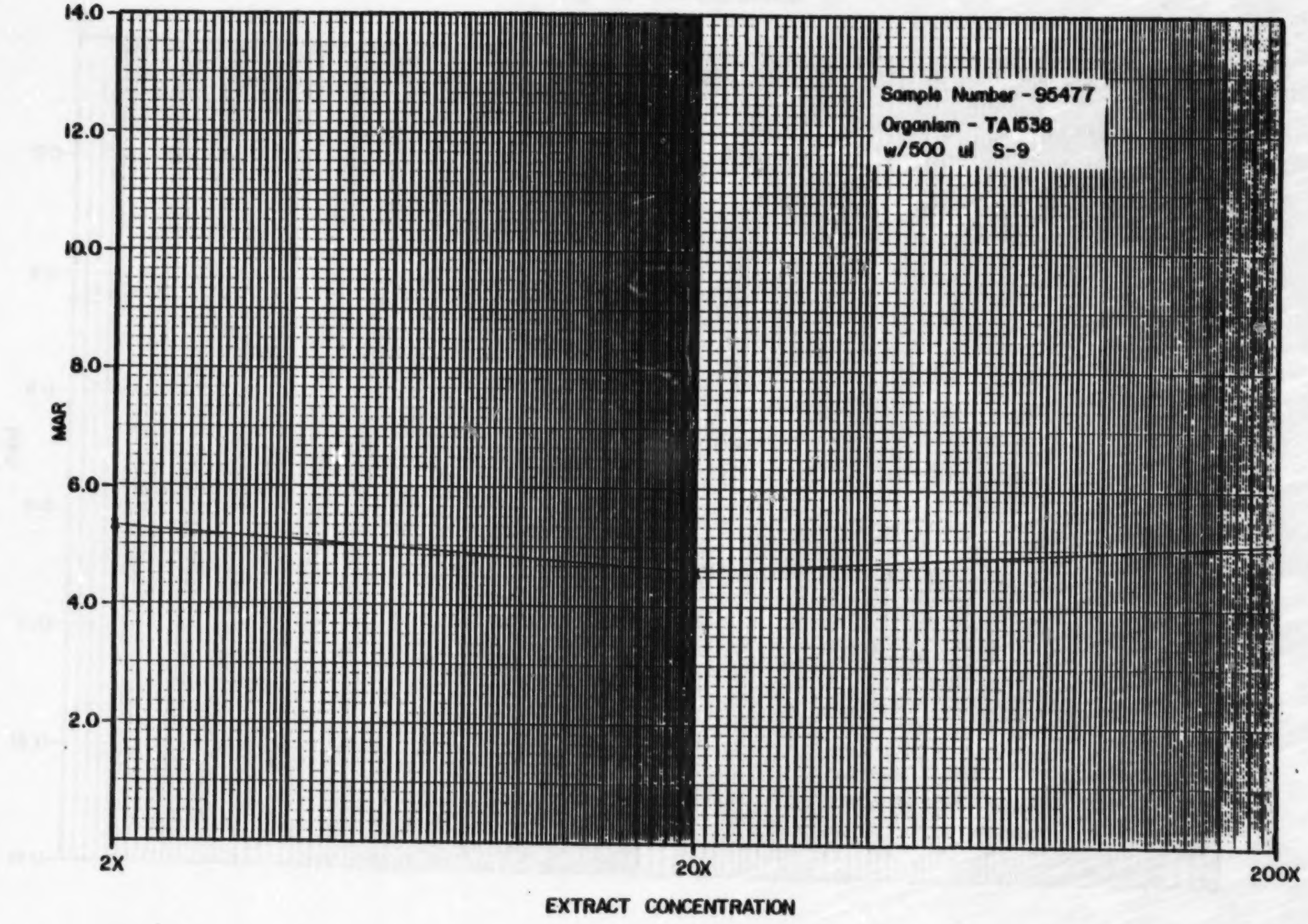

14.0
12.0
10.0
8.0
6.0
4.0
2.0
MAR
2X
20X
200X
EXTRACT CONCENTRATION
Sample Number - 95366
Organism - TA 1538

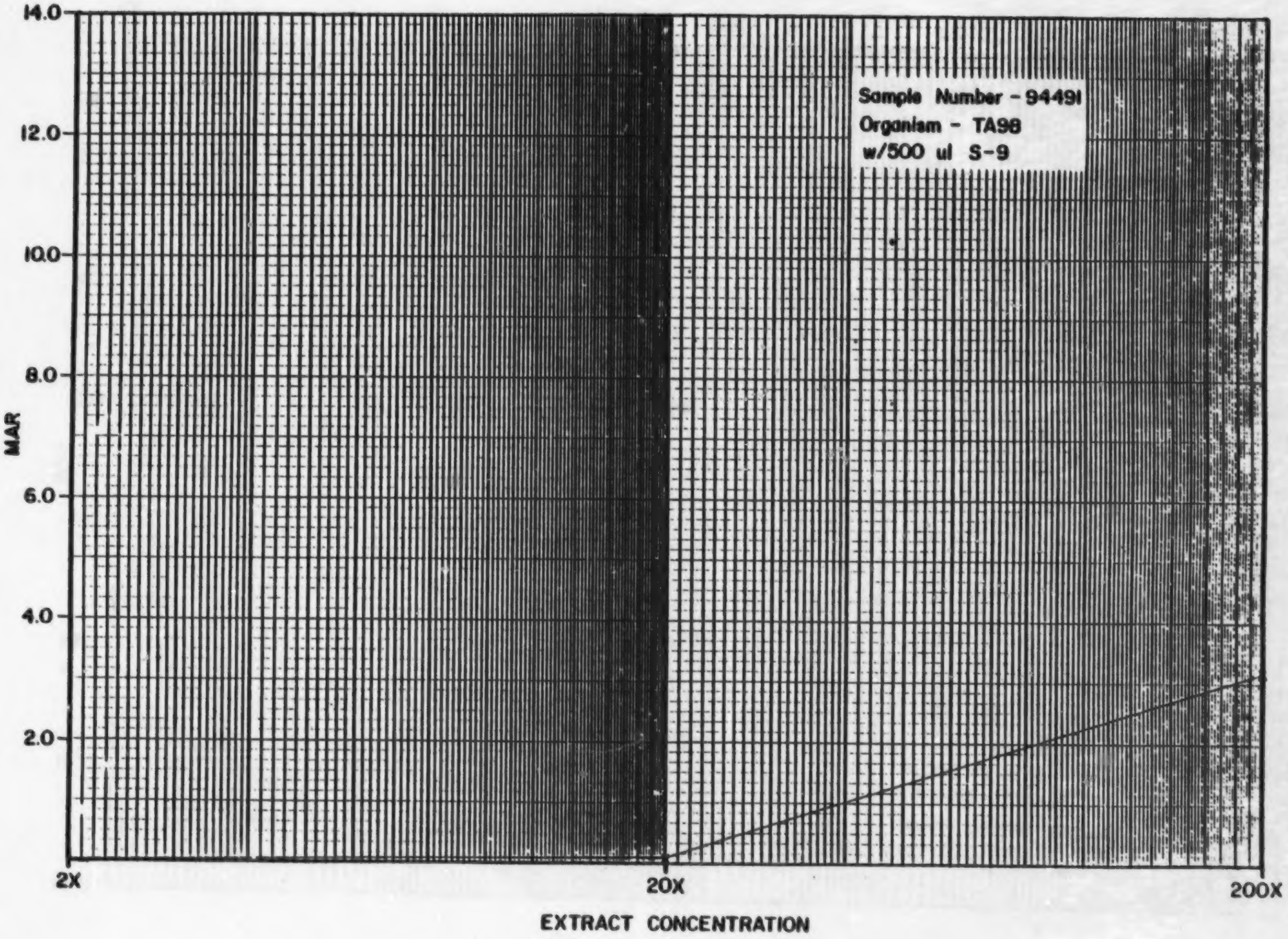

Sample Number - 95368
Organism - TA1538
MAR
14.0
12.0
10.0
8.0
6.0
4.0
2.0
2X
20X
200X
EXTRACT CONCENTRATION

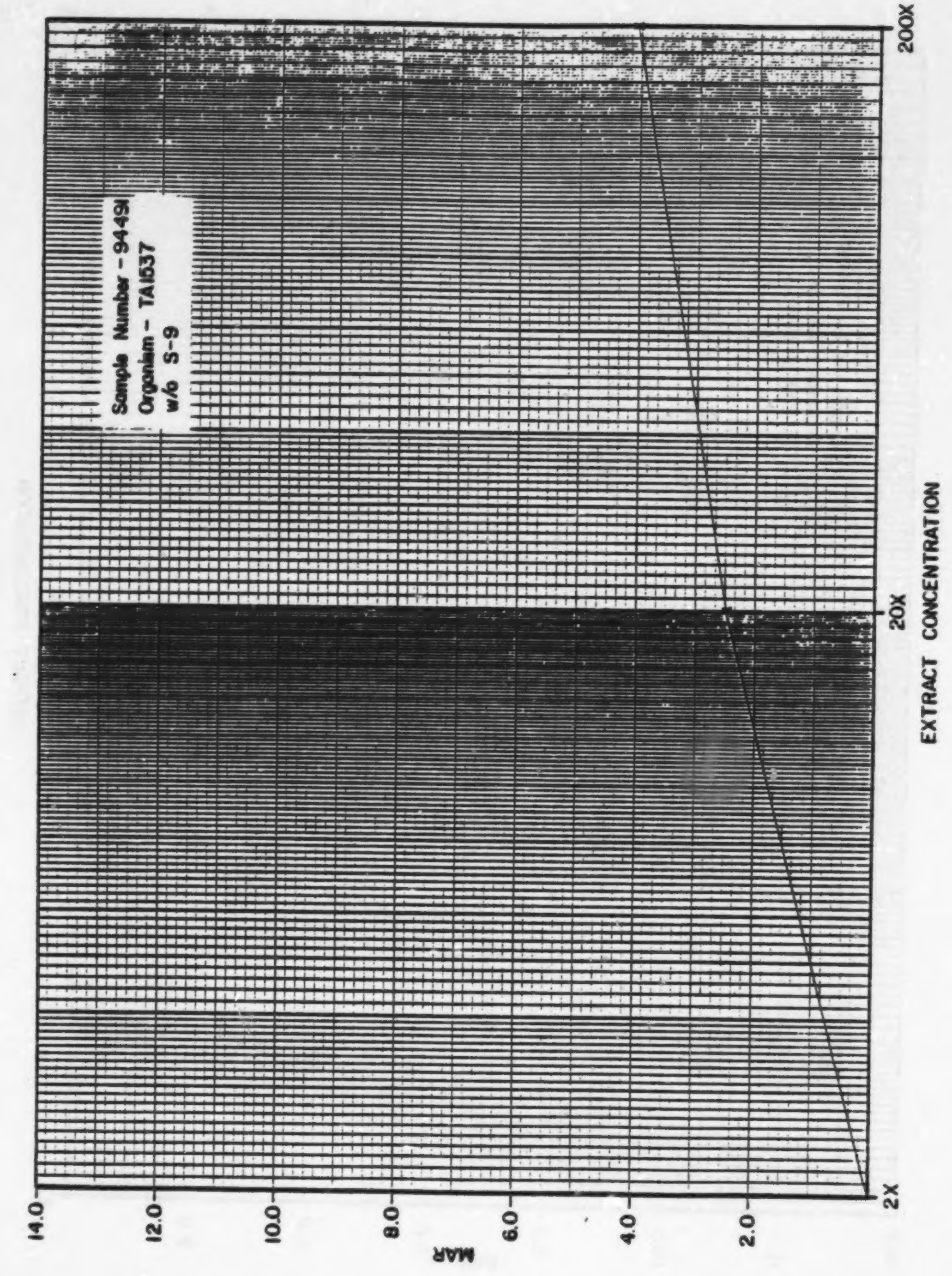

Sample Number - 95475
Organism - TA1538
MAR
14.0
12.0
10.0
8.0
6.0
4.0
2.0
2X
20X
200X
EXTRACT CONCENTRATION

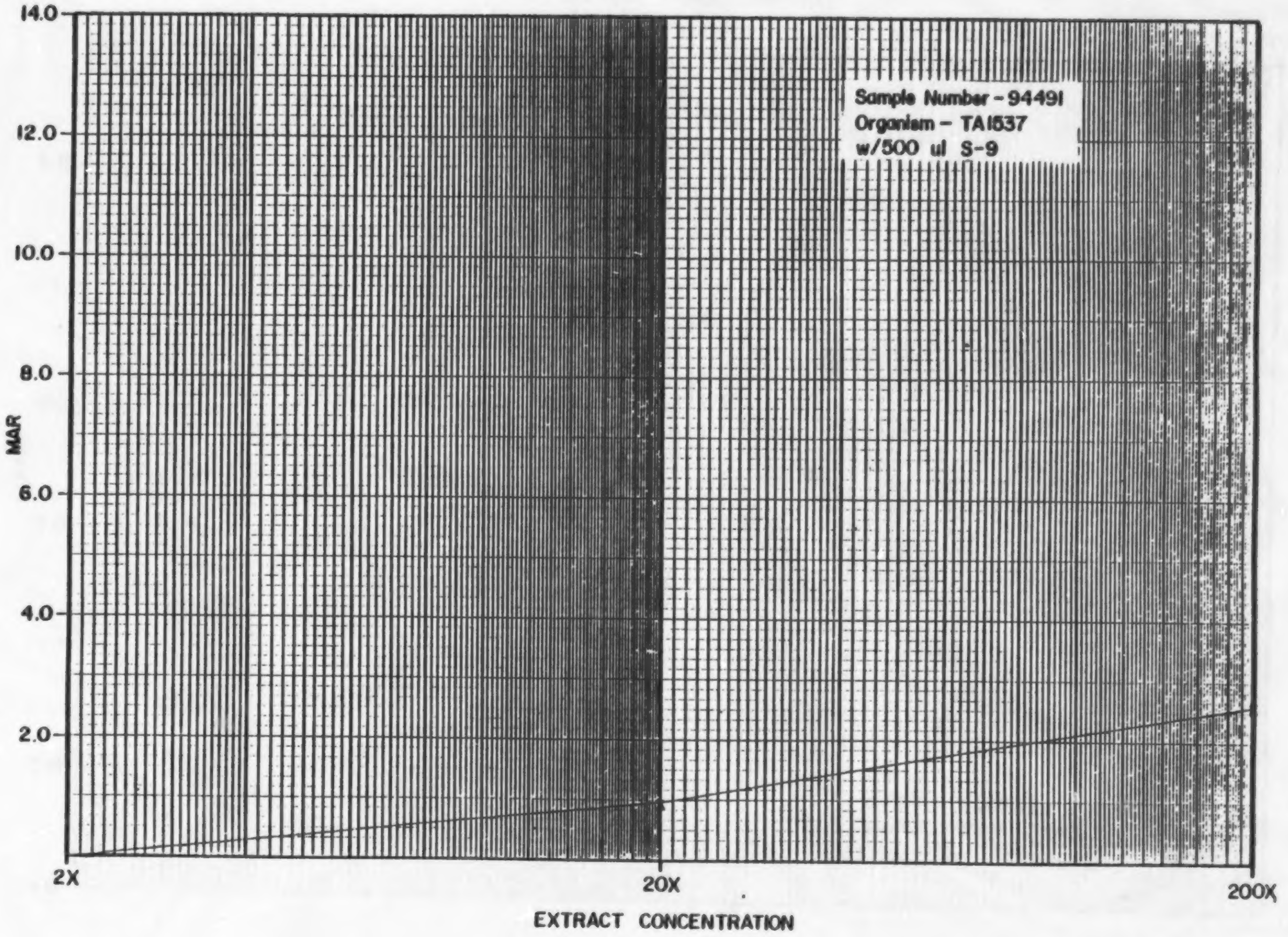

Sample Number - 95476
Organism - TA1538
MAR
14.0
12.0
10.0
8.0
6.0
4.0
2.0
2X
20X
200X
EXTRACT CONCENTRATION

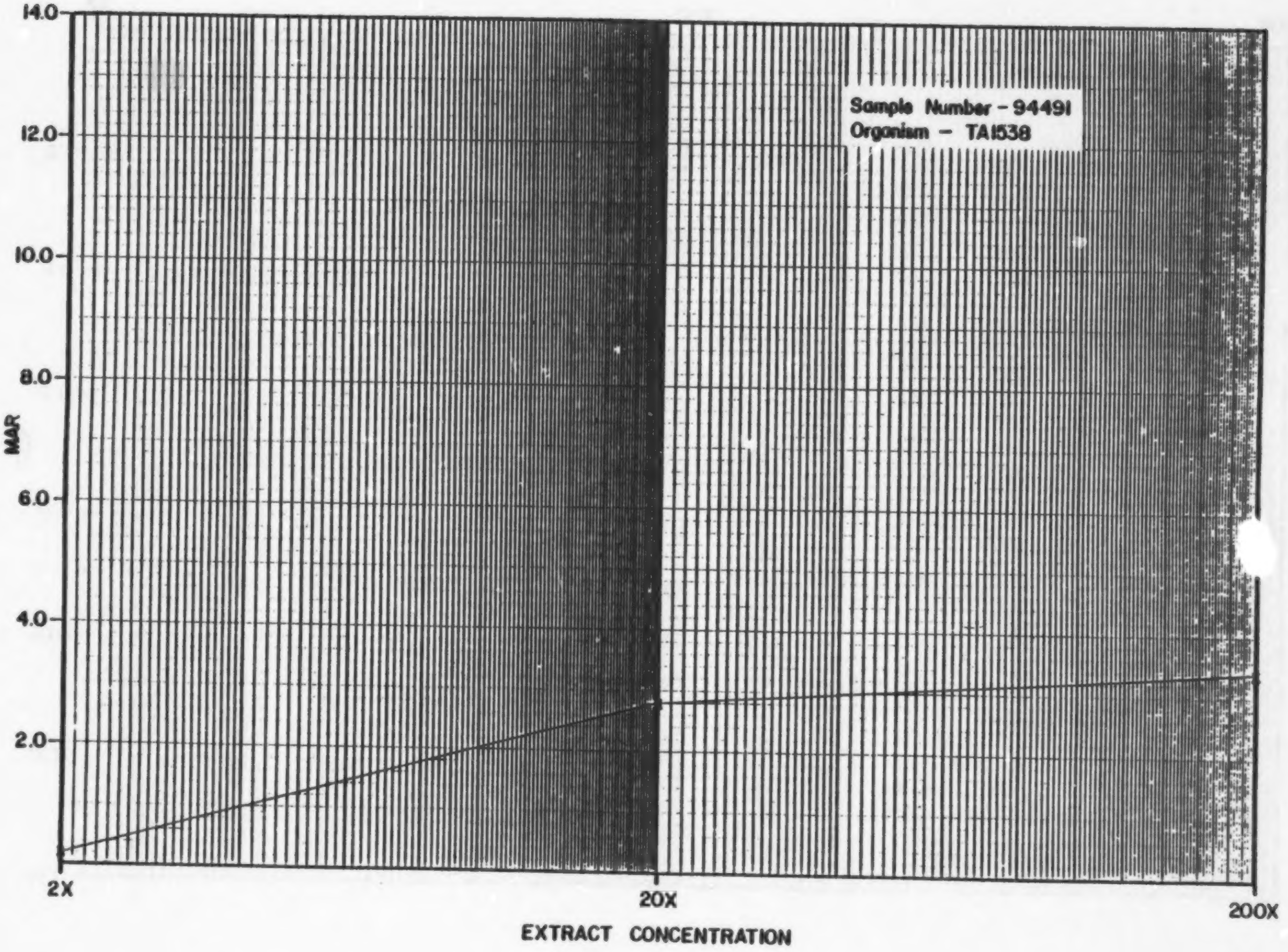

Sample Number - 95477
Organism - TA100
w/500 ul S-9
MAR
14.0
12.0
10.0
8.0
6.0
4.0
2.0
2X
20X
200X
EXTRACT CONCENTRATION

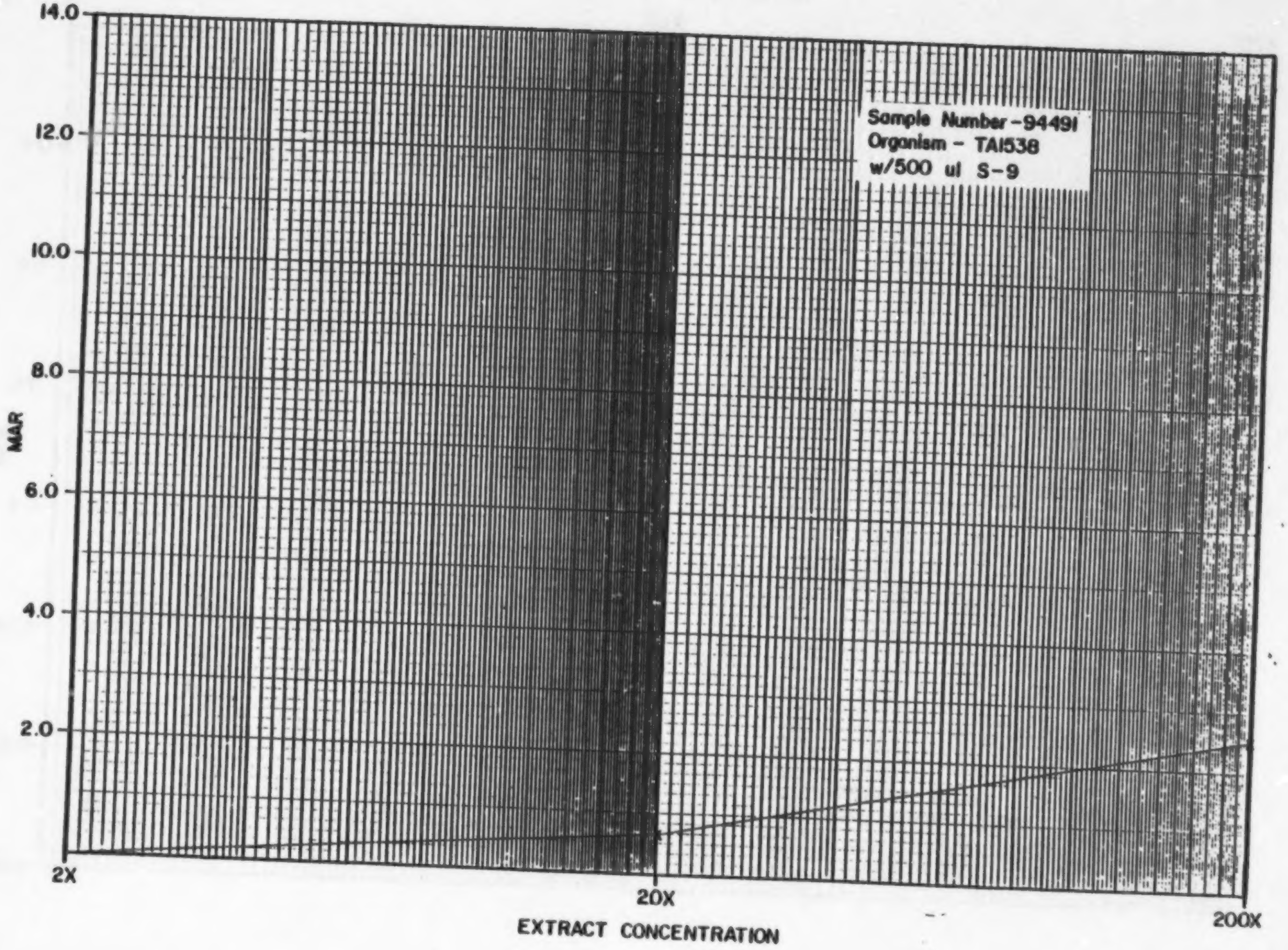

Sample Number - 95477
Organism - TA1538
w/500 ul S-9
MAR
14.0
12.0
10.0
8.0
6.0
4.0
2.0
2X
20X
200X
EXTRACT CONCENTRATION

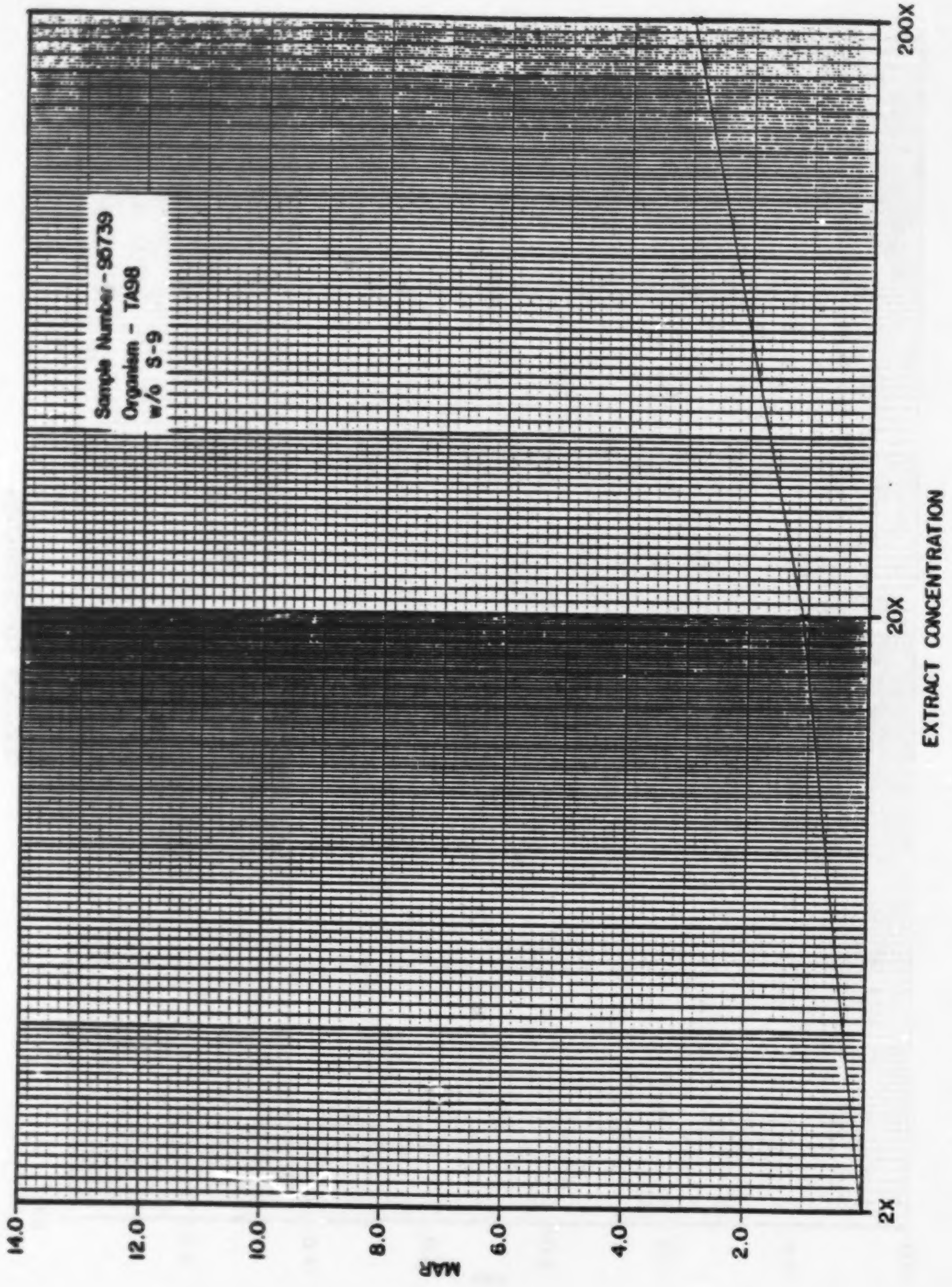

Sample Number - 94491
Organism - TA98
w/500 ul S-9
MAR
14.0
12.0
10.0
8.0
6.0
4.0
2.0
2X
20X
200X
EXTRACT CONCENTRATION

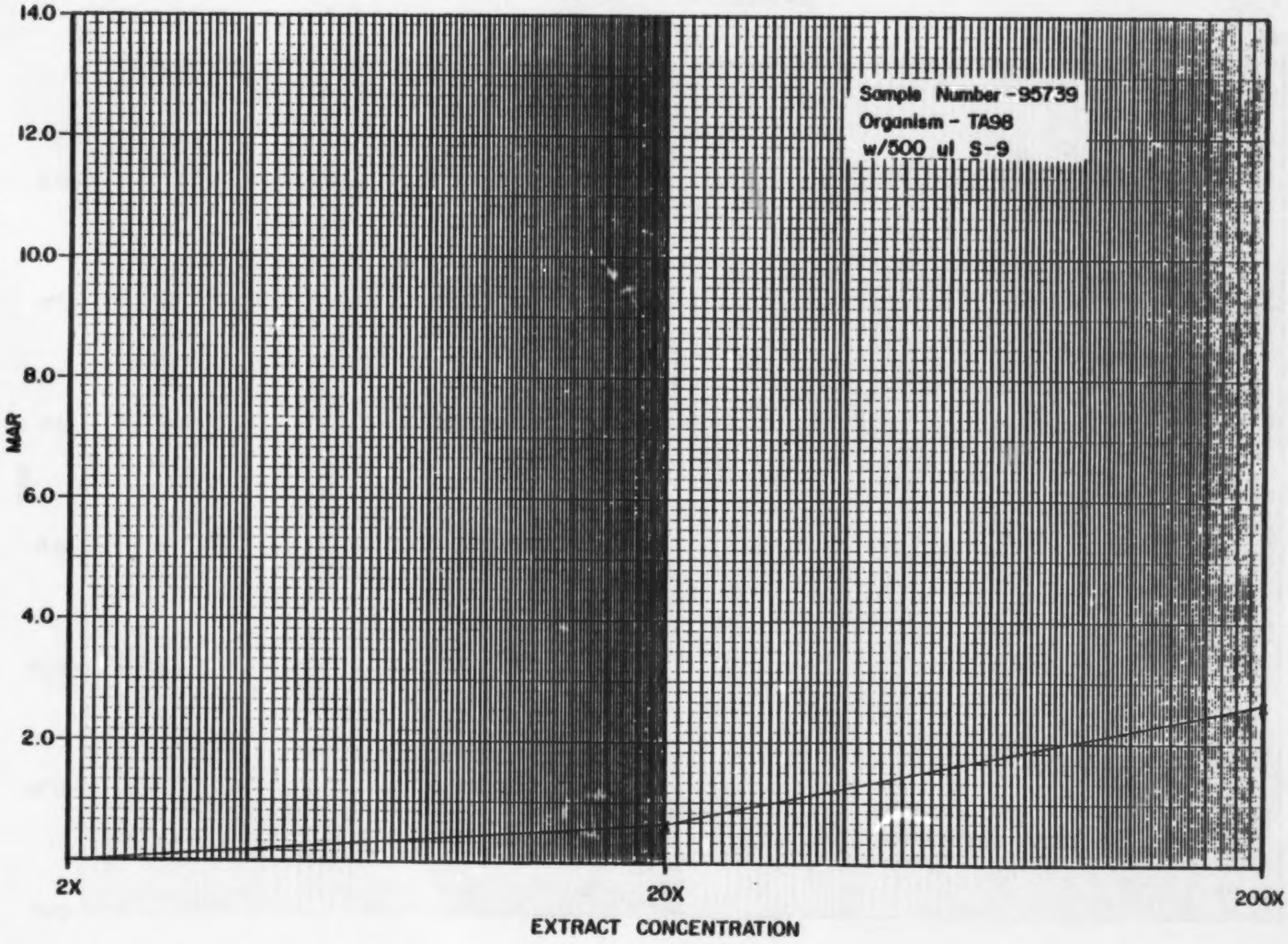

Sample Number - 94491
Organism - TA1537
w/o S-9
MAR
14.0
12.0
10.0
8.0
6.0
4.0
2.0
2X
20X
200X
EXTRACT CONCENTRATION

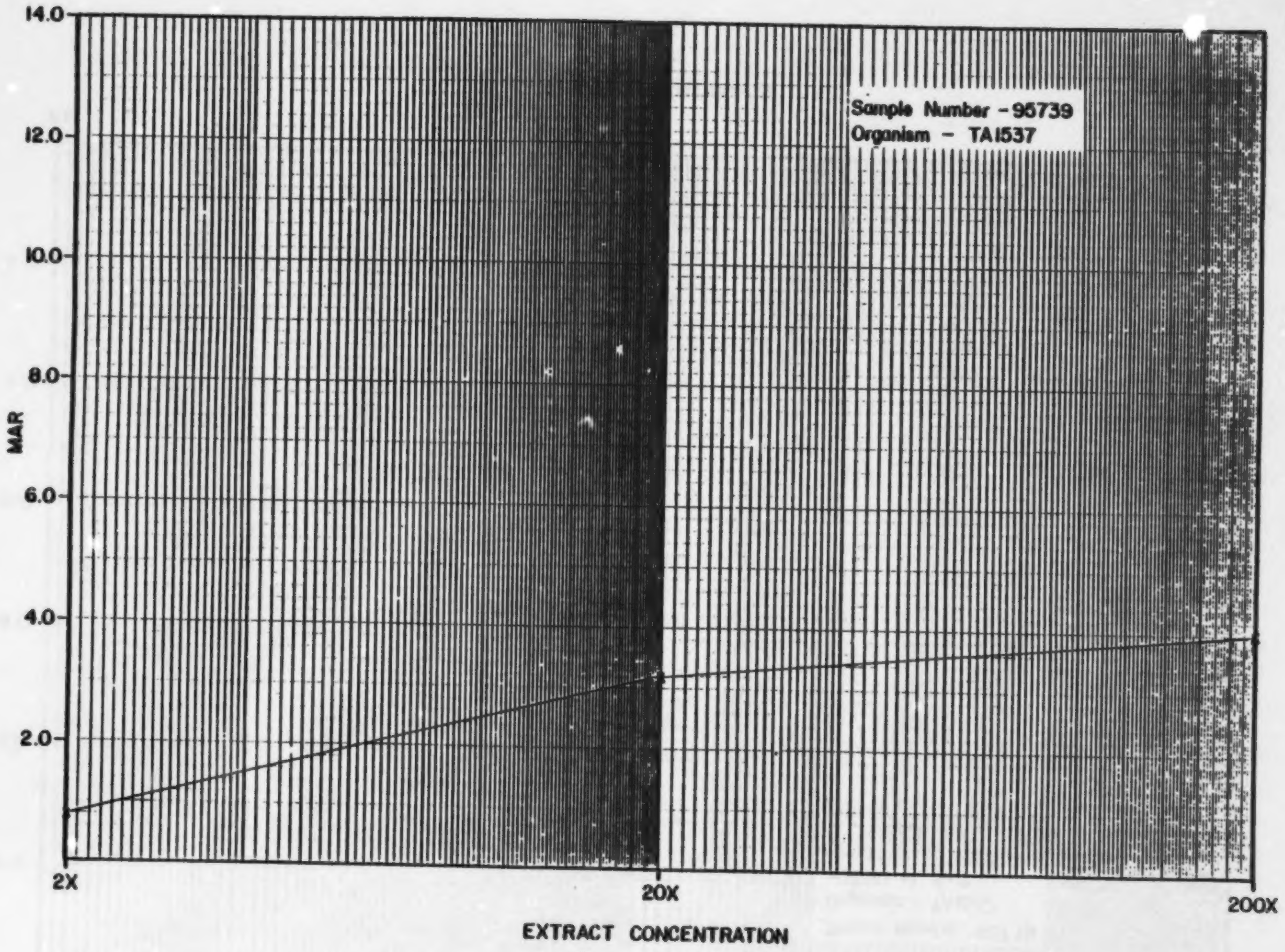

Sample Number - 94491
Organism - TA1537
w/500 ul S-9
14.0
12.0
10.0
8.0
6.0
4.0
2.0
MAR
2X
20X
200X
EXTRACT CONCENTRATION

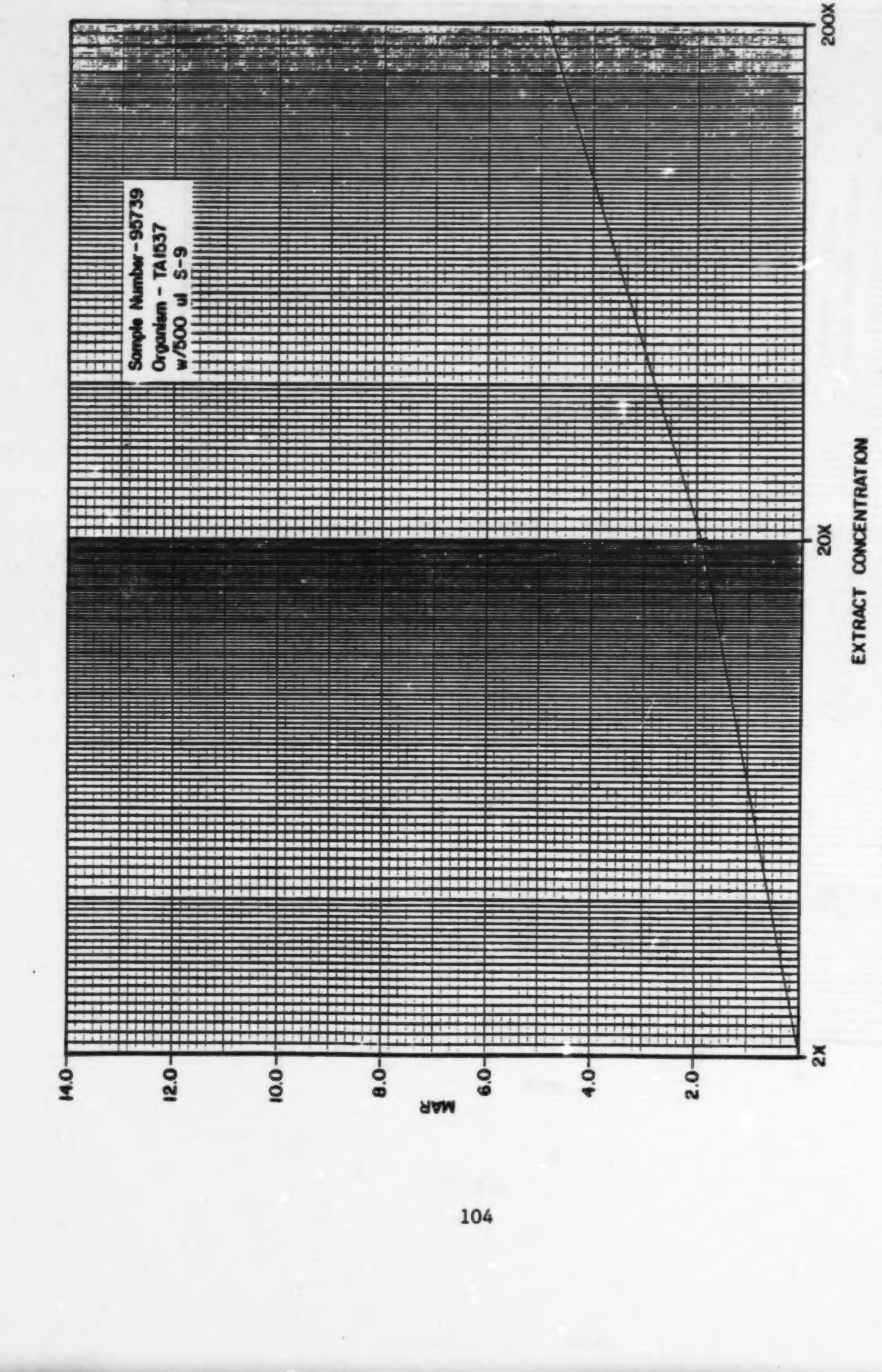

Sample Number - 94491
Organism - TA1538
14.0
12.0
10.0
8.0
6.0
4.0
2.0
MAR
2X
20X
200X
EXTRACT CONCENTRATION

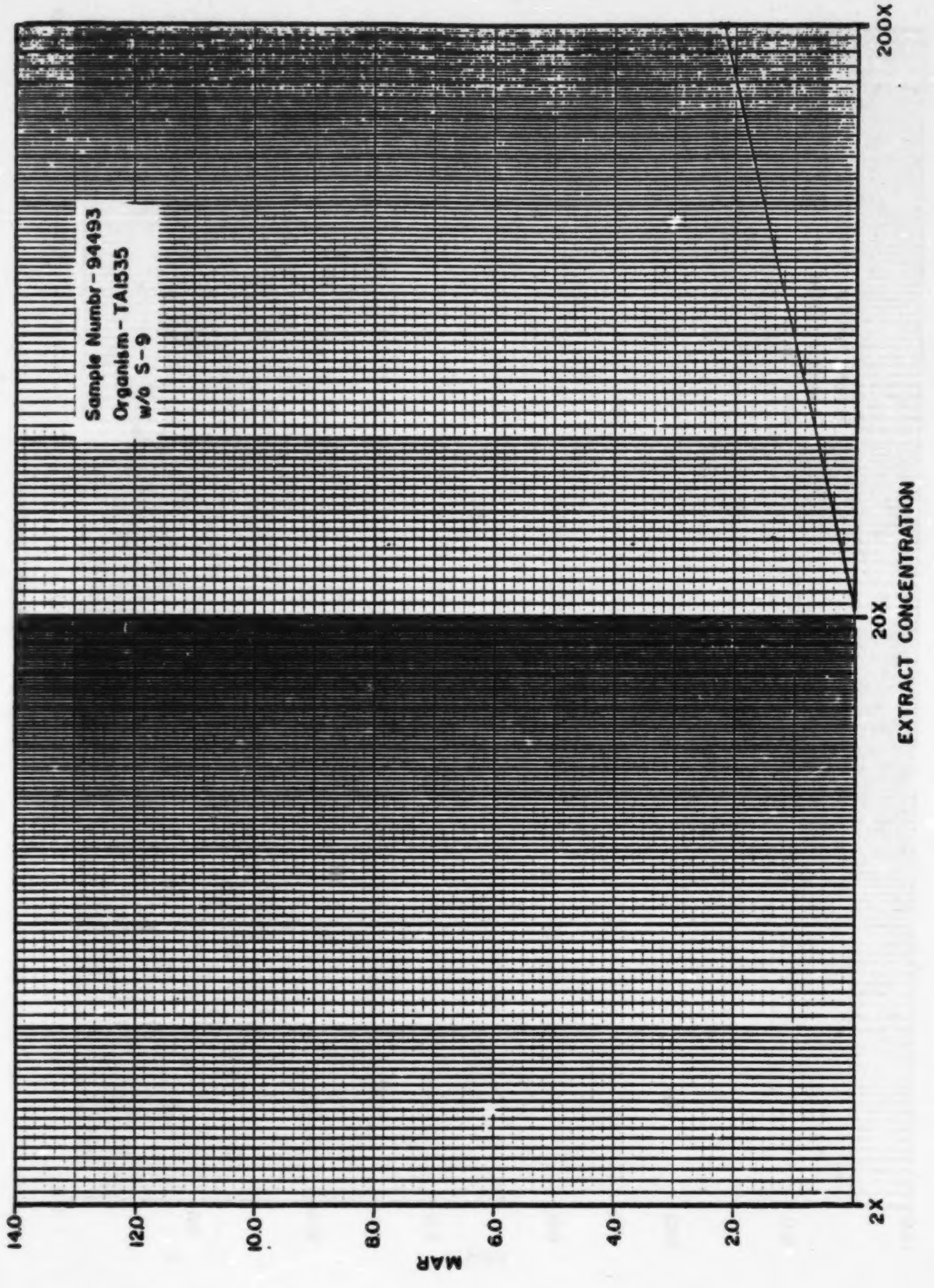

Sample Number-94491
Organism - TA1538
w/500 ul S-9
14.0
12.0
10.0
8.0
6.0
4.0
2.0
MAR
2X
20X
200X
EXTRACT CONCENTRATION

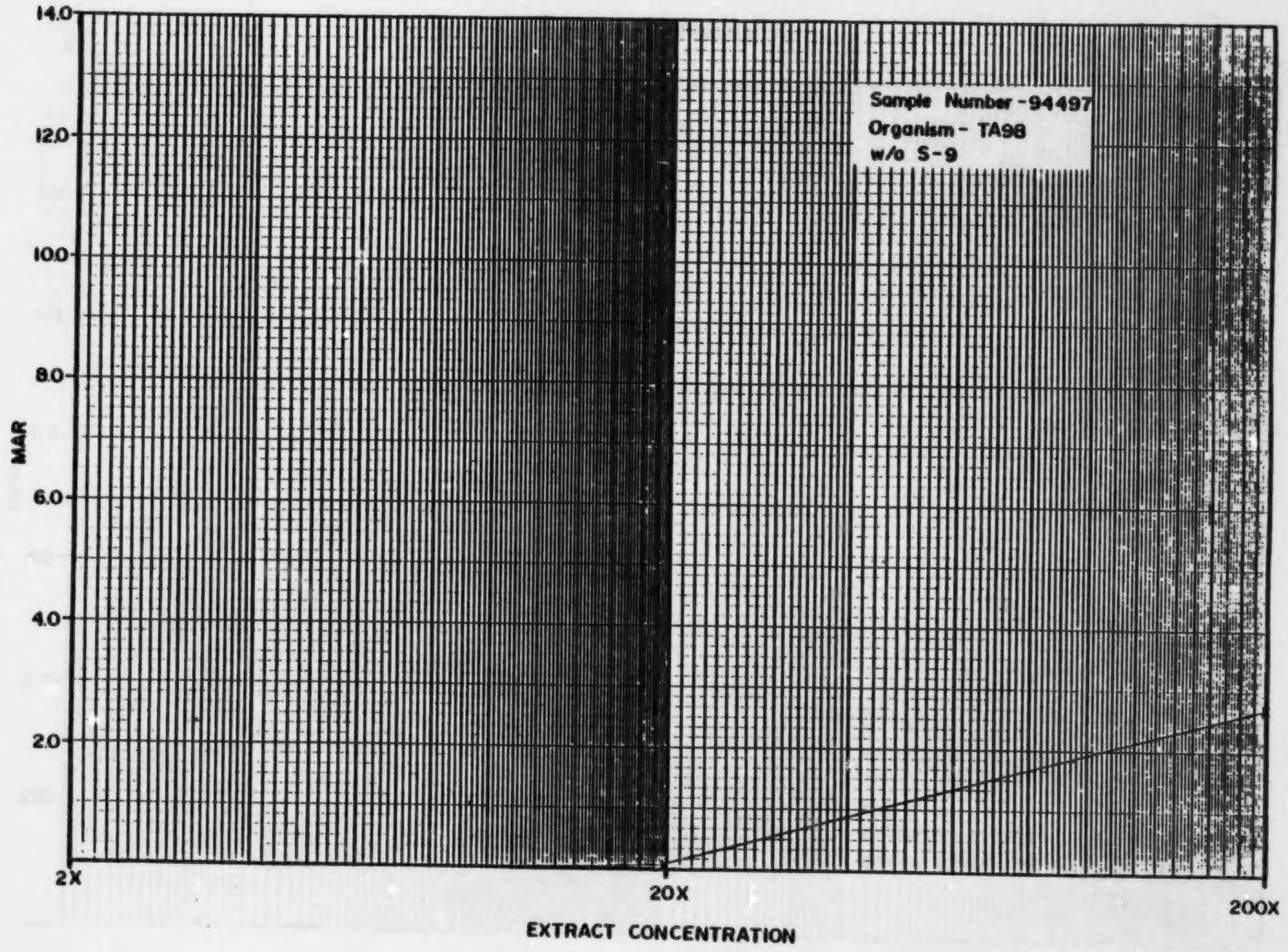

Sample Number - 95739
Organism - TA98
w/o S-9
14.0
12.0
10.0
8.0
6.0
4.0
2.0
MAR
2X
20X
200X
EXTRACT CONCENTRATION

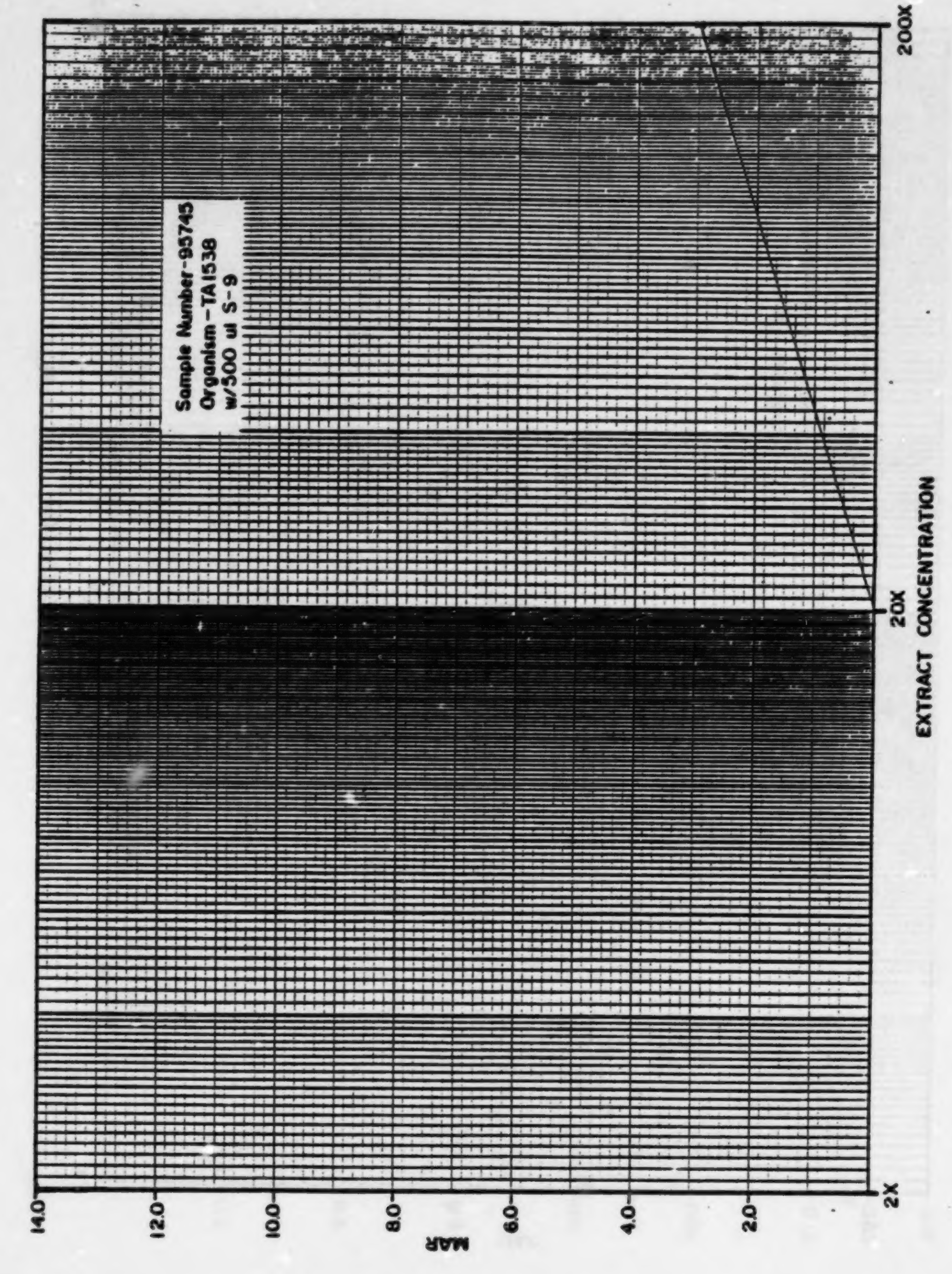

Sample Number - 95739
Organism - TA98
w/500 ul S-9
MAR
14.0
12.0
10.0
8.0
6.0
4.0
2.0
2X
20X
200X
EXTRACT CONCENTRATION

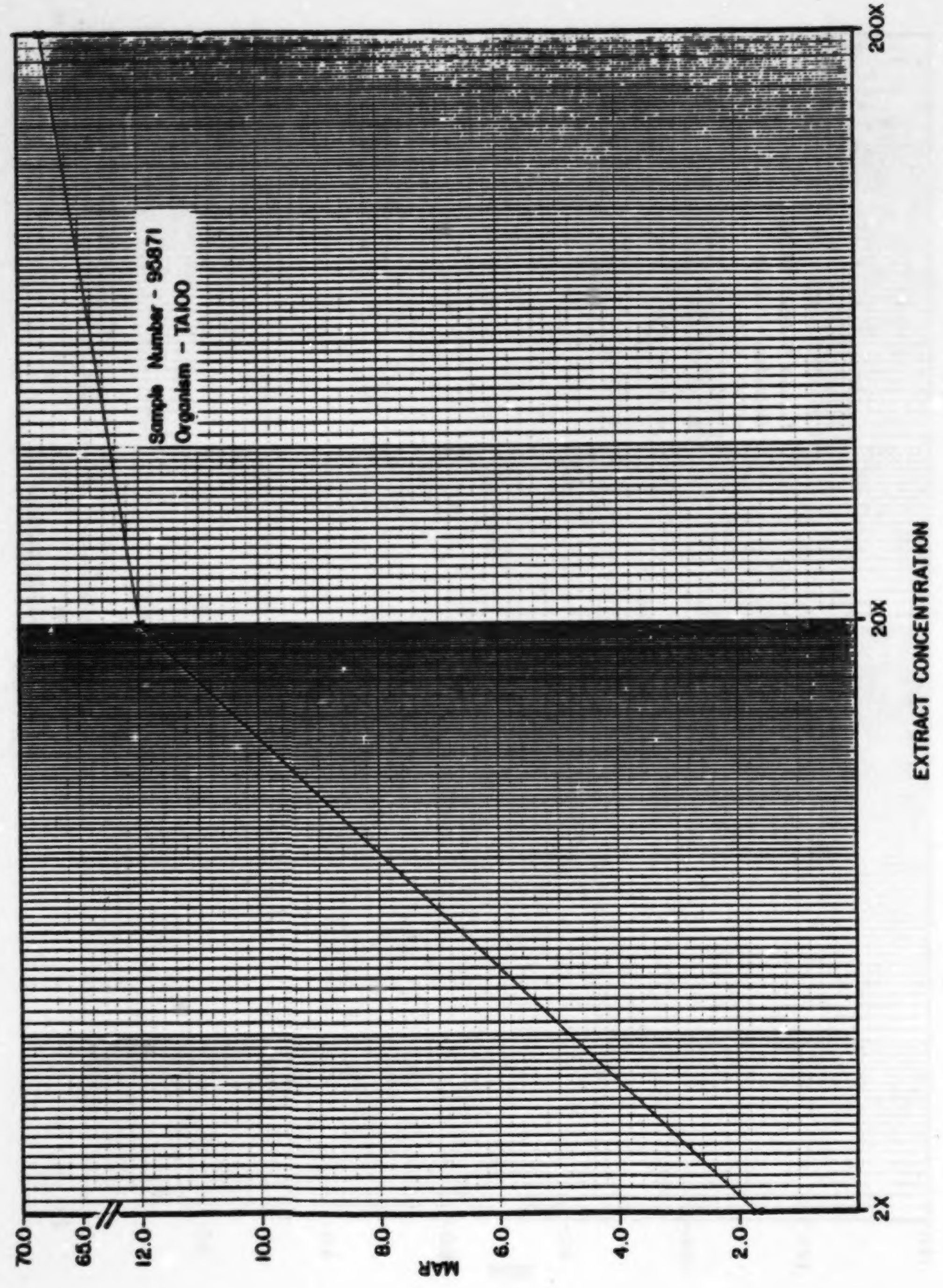

Sample Number - 95739
Organism - TA1537
14.0
12.0
10.0
8.0
6.0
4.0
2.0
MAR
2X
20X
200X
EXTRACT CONCENTRATION

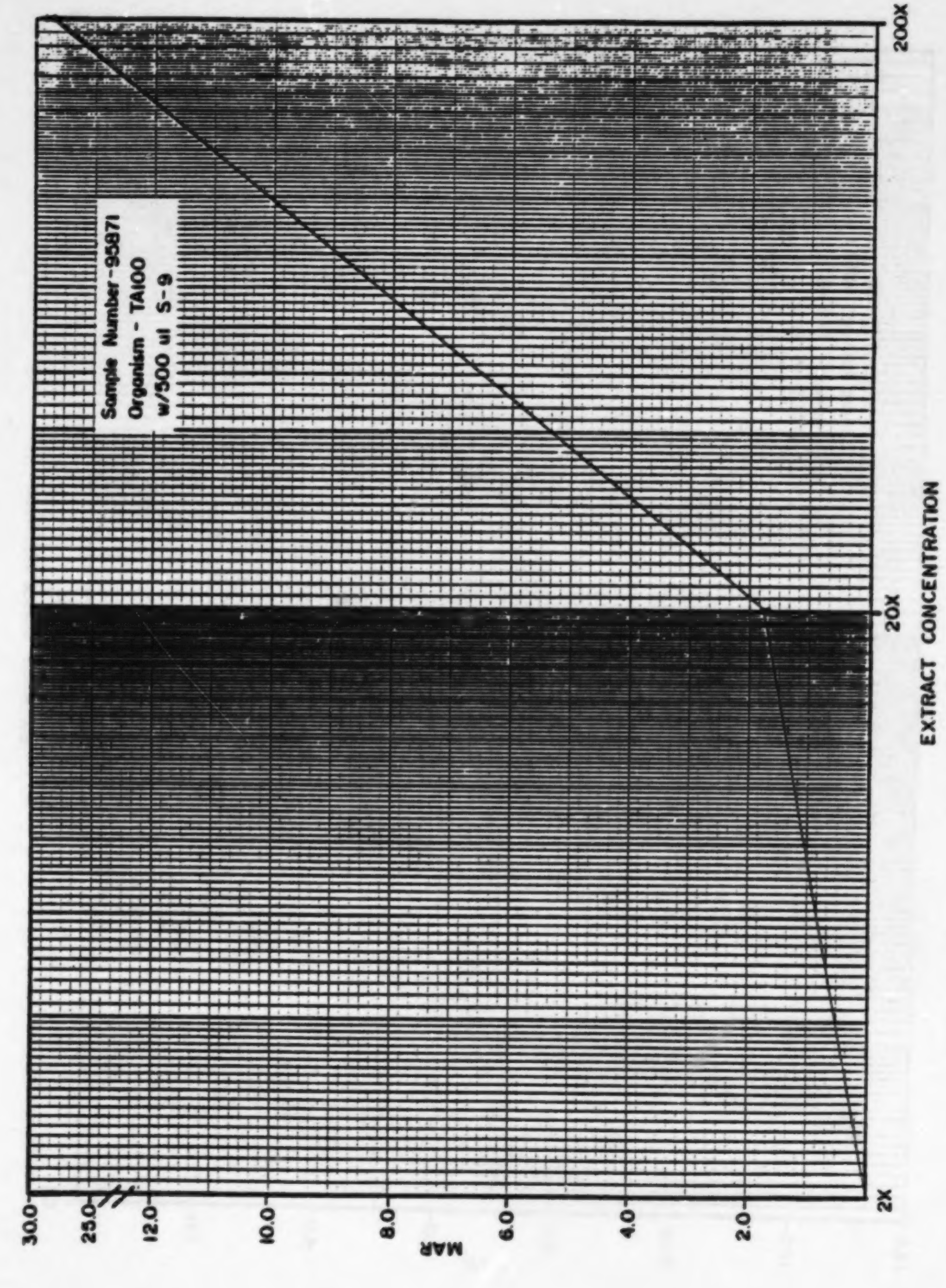

Sample Number - 95739
Organism - TA1537
w/500 ul S-9
MAR
14.0
12.0
10.0
8.0
6.0
4.0
2.0
2X
20X
200X
EXTRACT CONCENTRATION

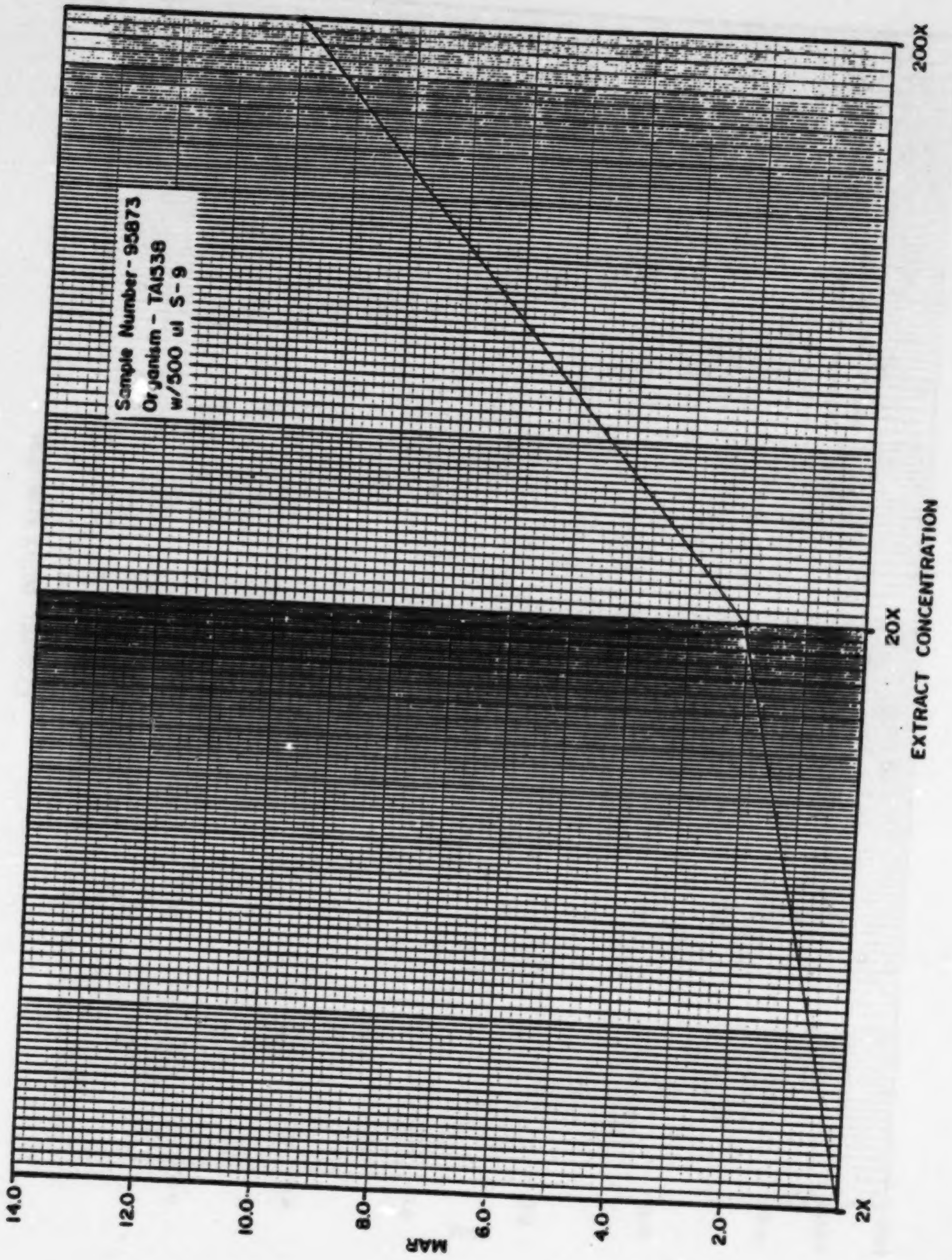

Sample Numbr - 94493
Organism - TA1535
w/o S-9
MAR
14.0
12.0
10.0
8.0
6.0
4.0
2.0
2X
20X
200X
EXTRACT CONCENTRATION

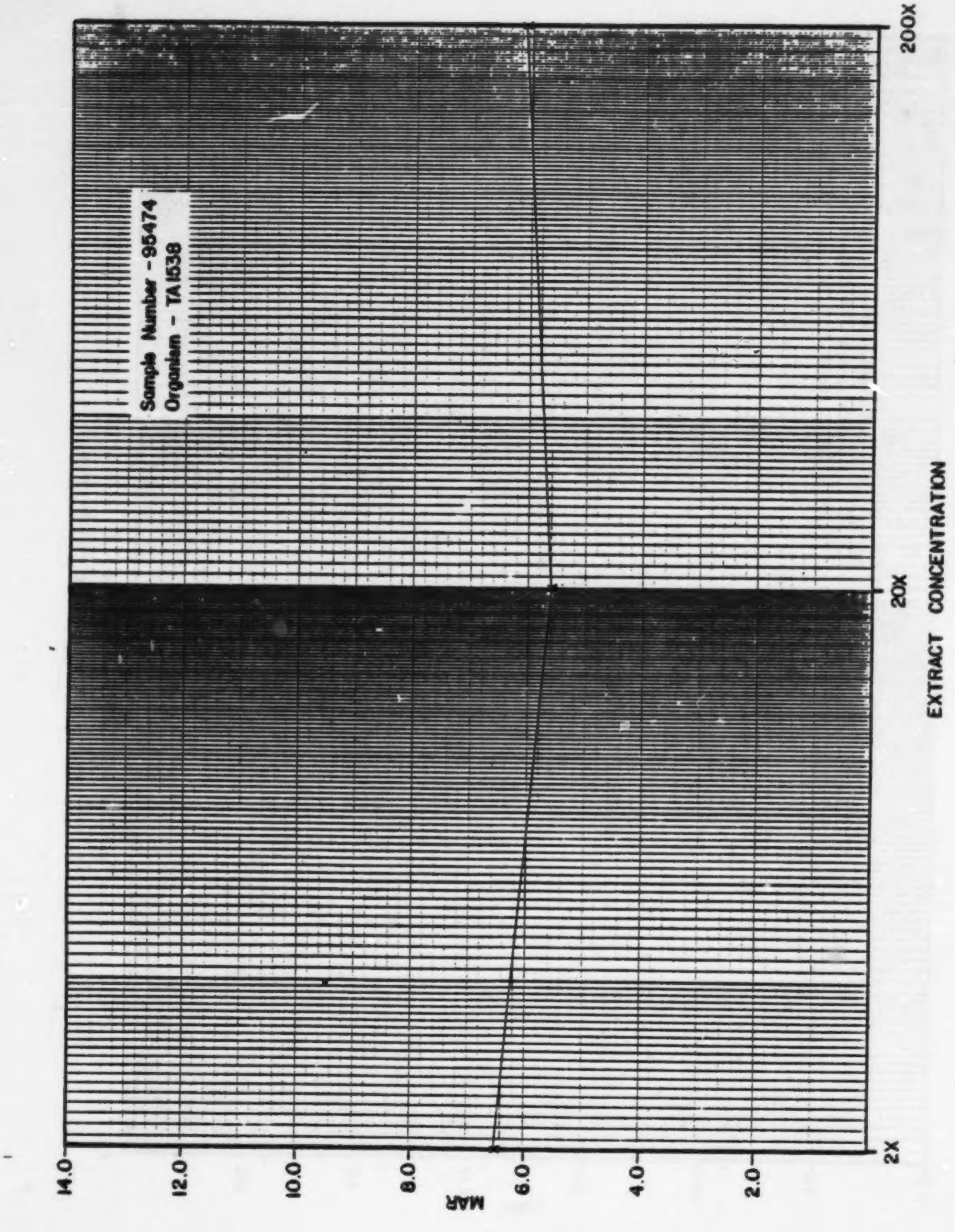

Sample Number-94497
Organism- TA98
w/o S-9
14.0
12.0
10.0
8.0
6.0
4.0
2.0
MAR
2X
20X
200X
EXTRACT CONCENTRATION


Sample Number-95745
Organism-TA1538
w/500 ul S-9
MAR
14.0
12.0
10.0
8.0
6.0
4.0
2.0
2X
20X
200X
EXTRACT CONCENTRATION

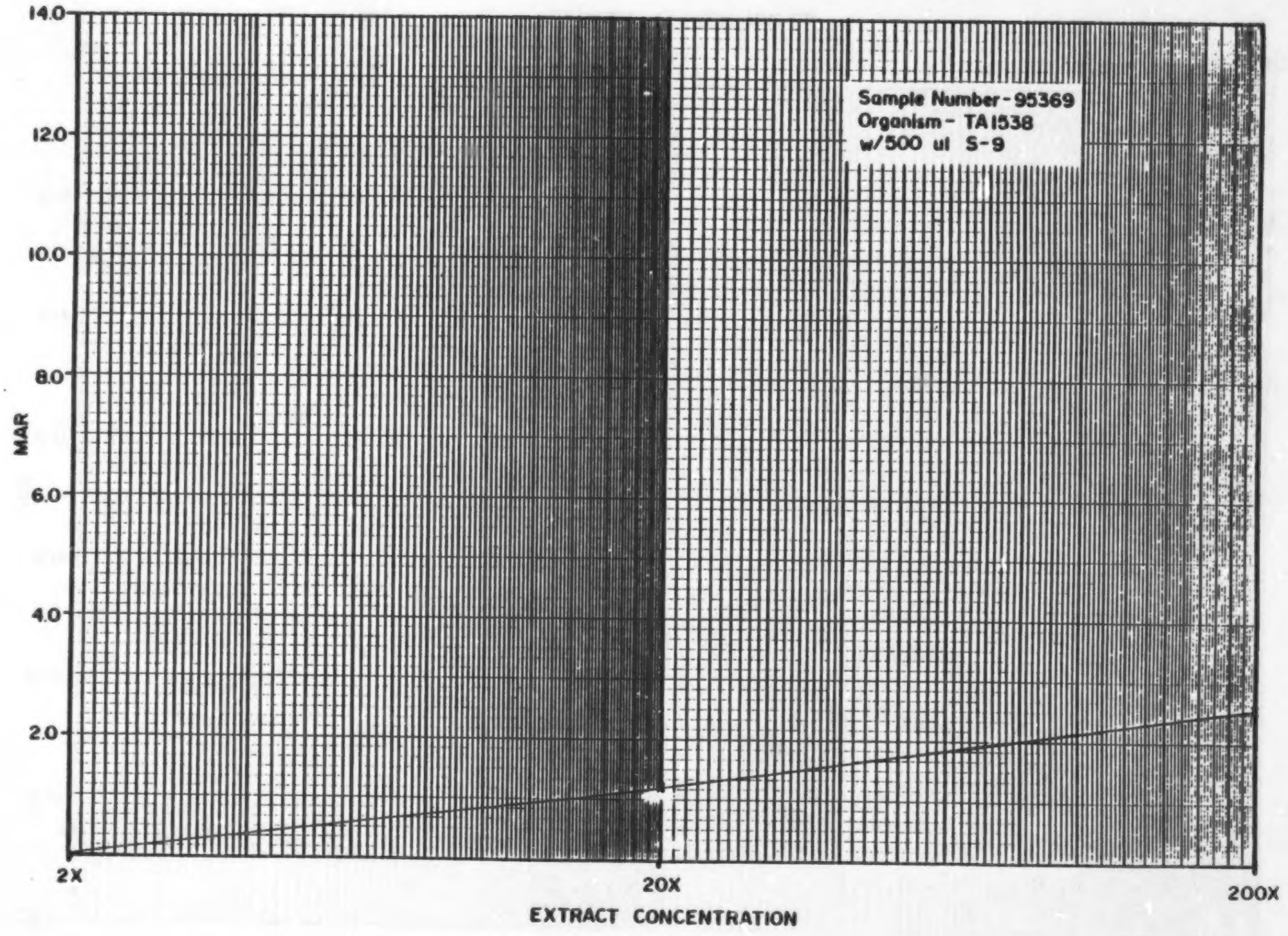

Sample Number - 95871
Organism - TA100
70.0
65.0
12.0
10.0
8.0
6.0
4.0
2.0
MAR
2X
20X
200X
EXTRACT CONCENTRATION

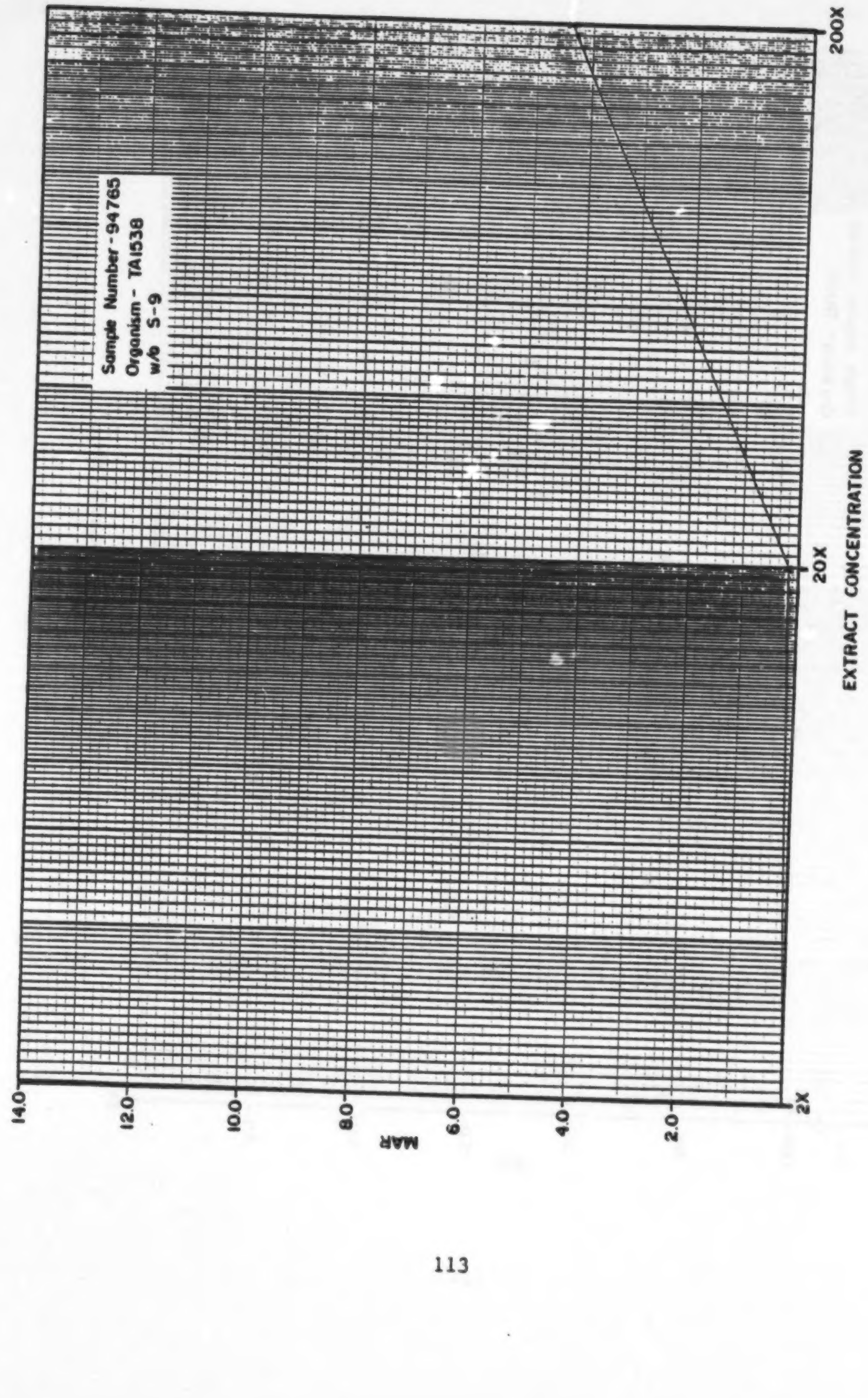

Sample Number-95871
Organism - TA100
w/500 ul S-9
MAR
30.0
25.0
12.0
10.0
8.0
6.0
4.0
2.0
2X
20X
200X
EXTRACT CONCENTRATION

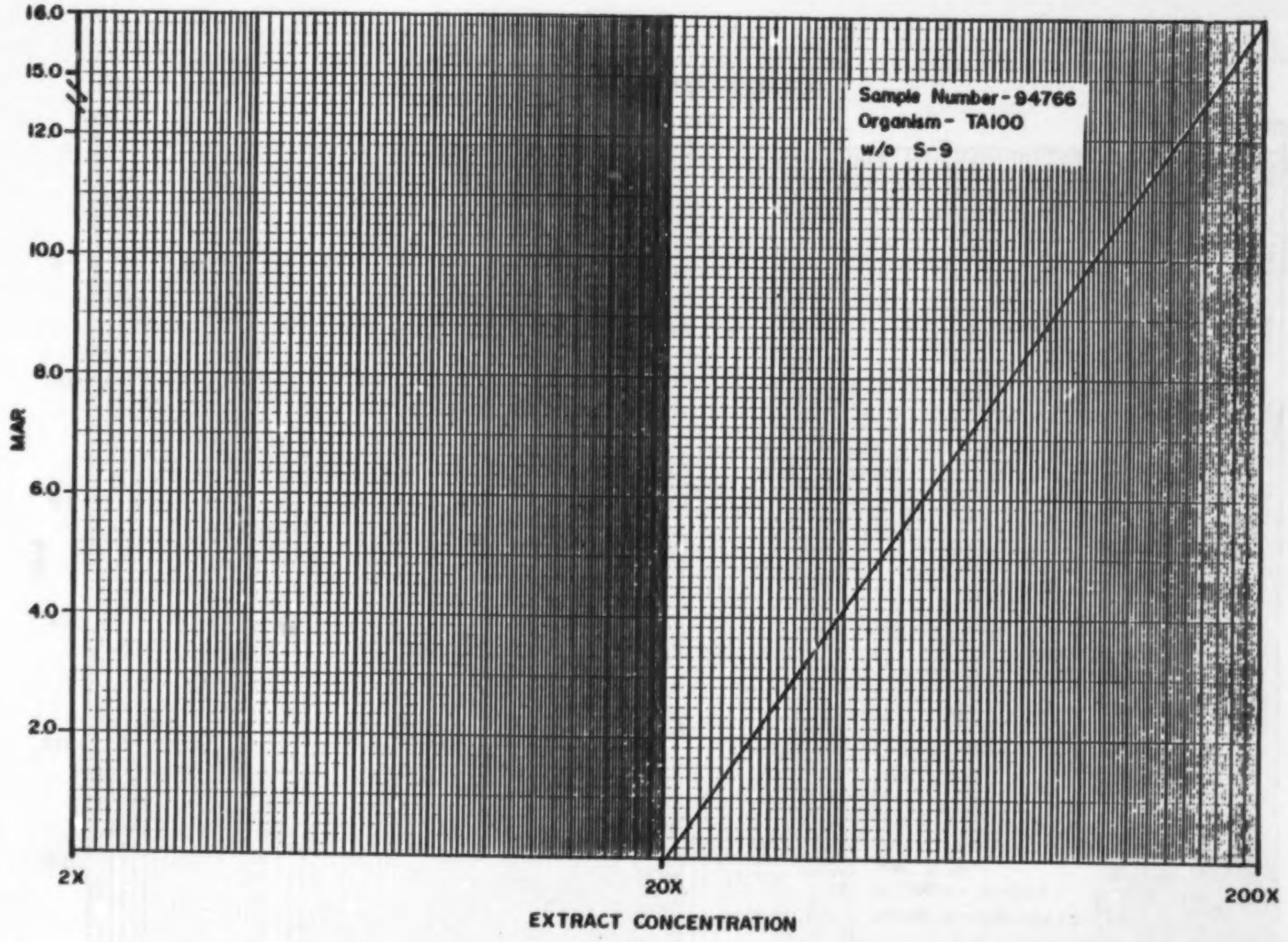

Sample Number - 95873
Organism - TA1538
w/500 ul S-9
MAR
14.0
12.0
10.0
8.0
6.0
4.0
2.0
2X
20X
200X
EXTRACT CONCENTRATION

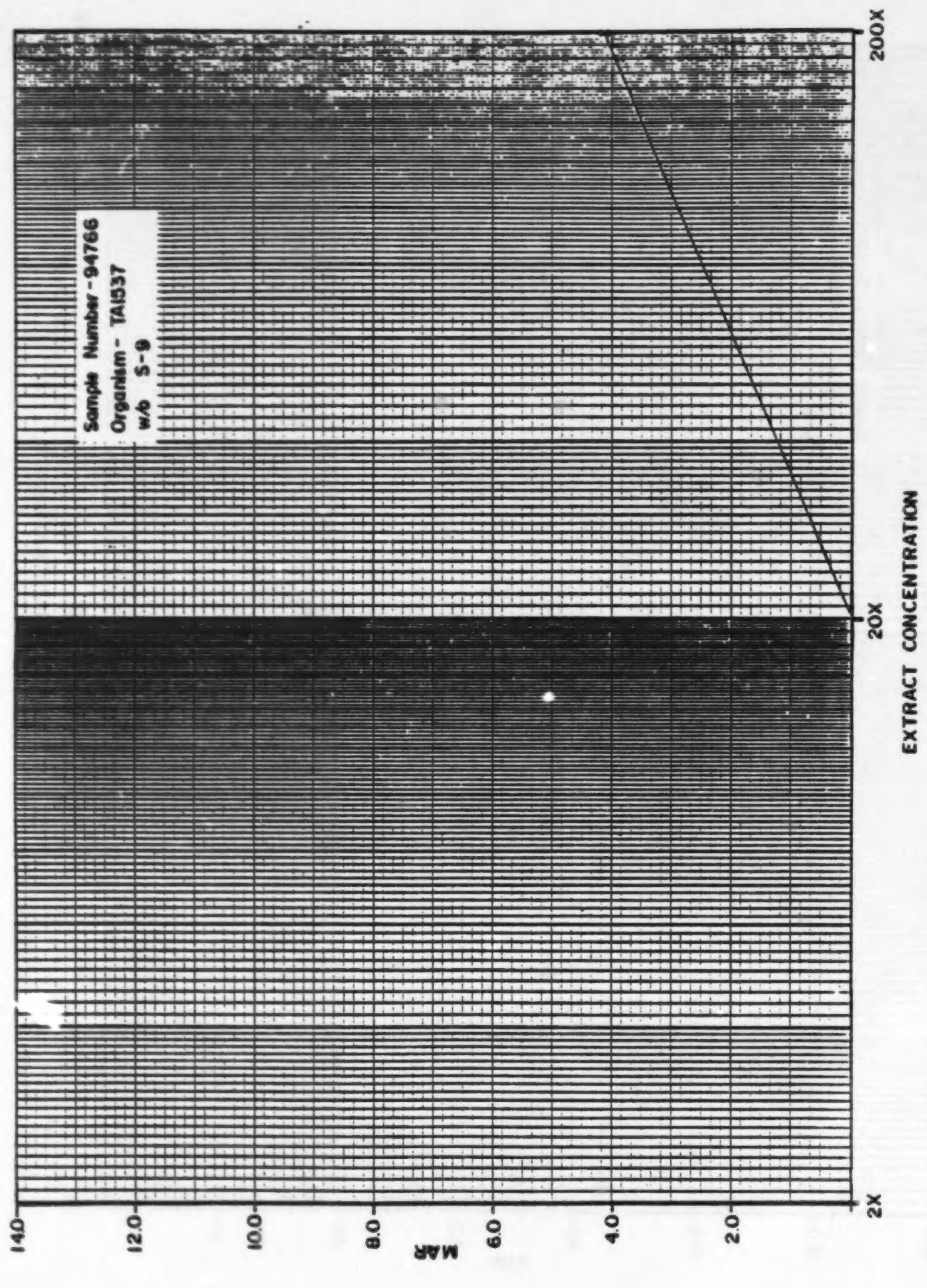


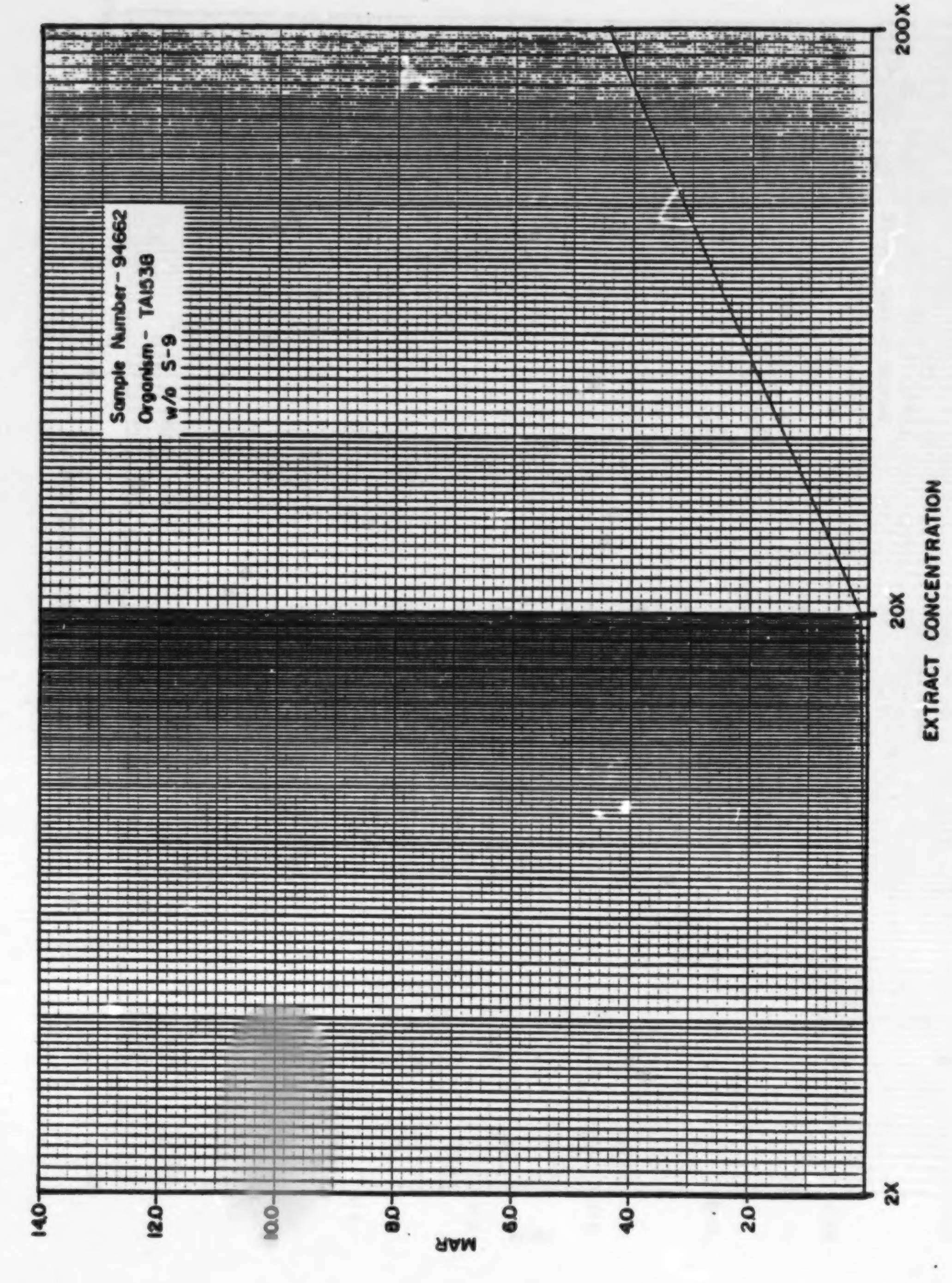
Sample Number - 95474
Organism - TA1538
MAR
14.0
12.0
10.0
8.0
6.0
4.0
2.0
2X
20X
200X
EXTRACT CONCENTRATION

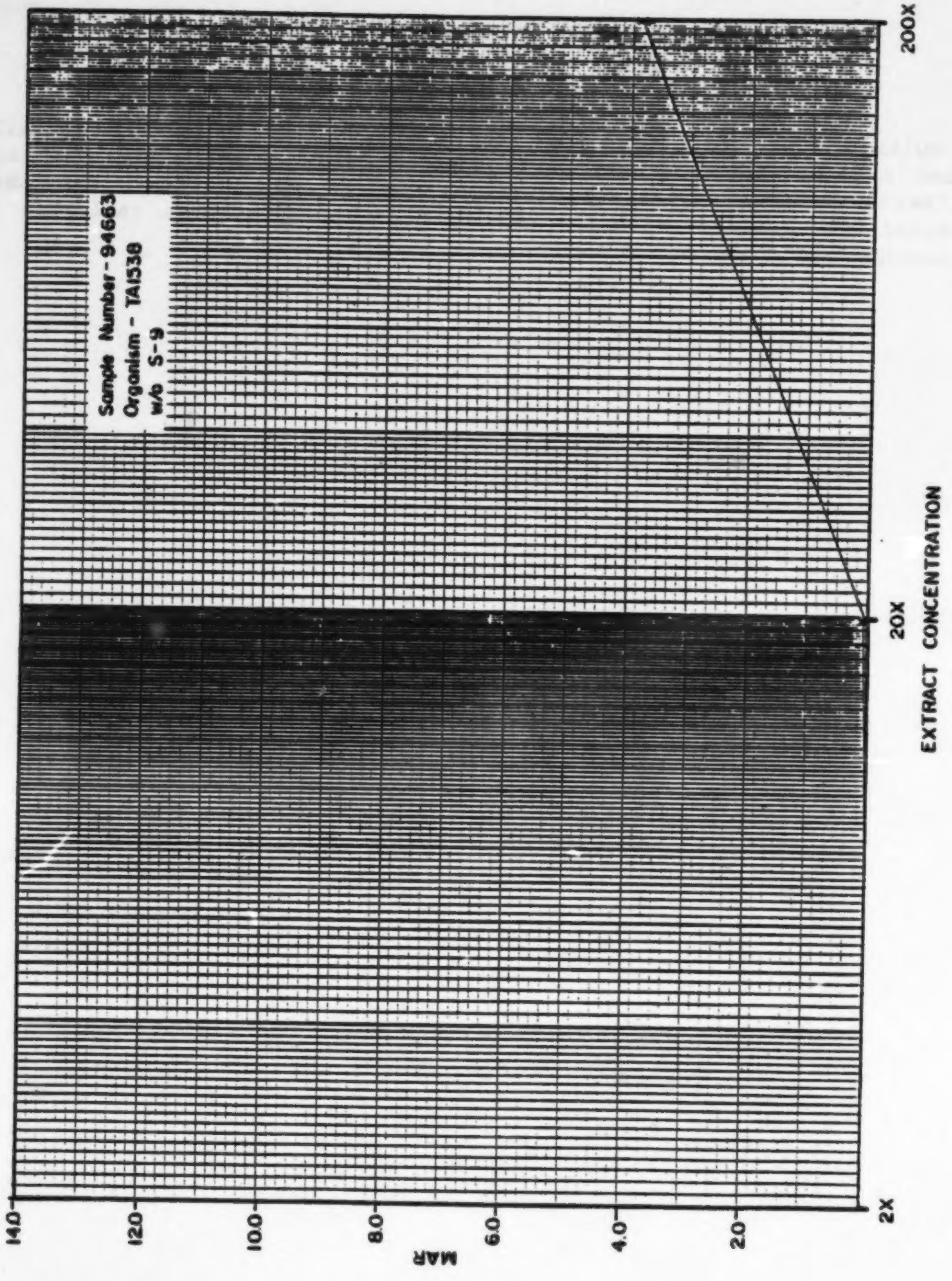

14.0
12.0
10.0
8.0
6.0
4.0
2.0
MAR
2X
20X
200X
EXTRACT CONCENTRATION
Sample Number - 95369
Organism - TA1538
w/500 ul S-9


Sample Number - 94765
Organism - TA1538
w/o S-9
MAR
14.0
12.0
10.0
8.0
6.0
4.0
2.0
2X
20X
200X
EXTRACT CONCENTRATION


16.0
15.0
12.0
10.0
8.0
6.0
4.0
2.0
MAR
2X
20X
200X
EXTRACT CONCENTRATION
Sample Number - 94766
Organism - TA100
w/o S-9


Sample Number - 94766
Organism - TA1537
w/o S-9
MAR
14.0
12.0
10.0
8.0
6.0
4.0
2.0
2X
20X
200X
EXTRACT CONCENTRATION

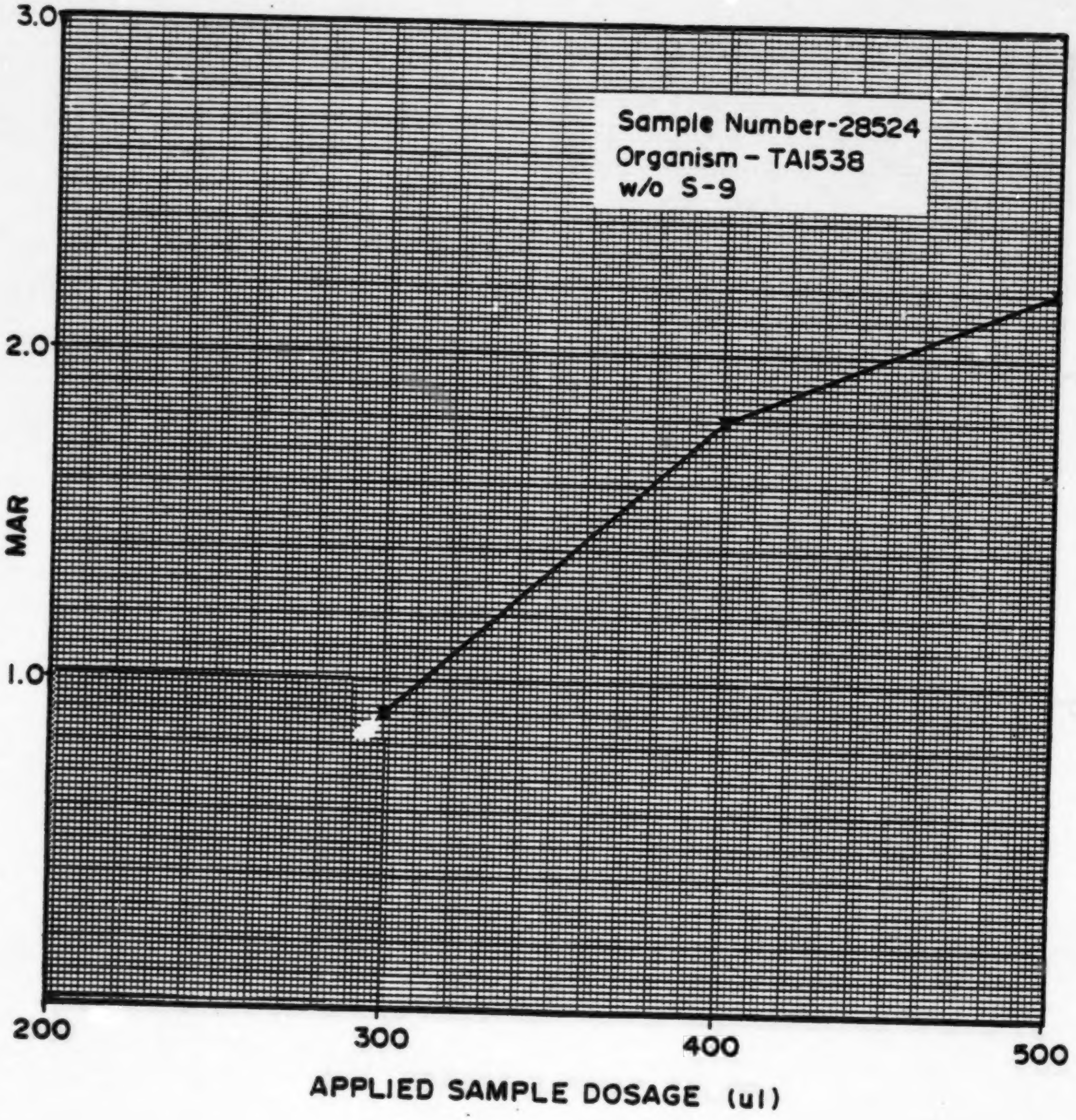

Sample Number - 94662
Organism - TA1538
w/o S-9
MAR
14.0
12.0
10.0
8.0
6.0
4.0
2.0
2X
20X
200X
EXTRACT CONCENTRATION

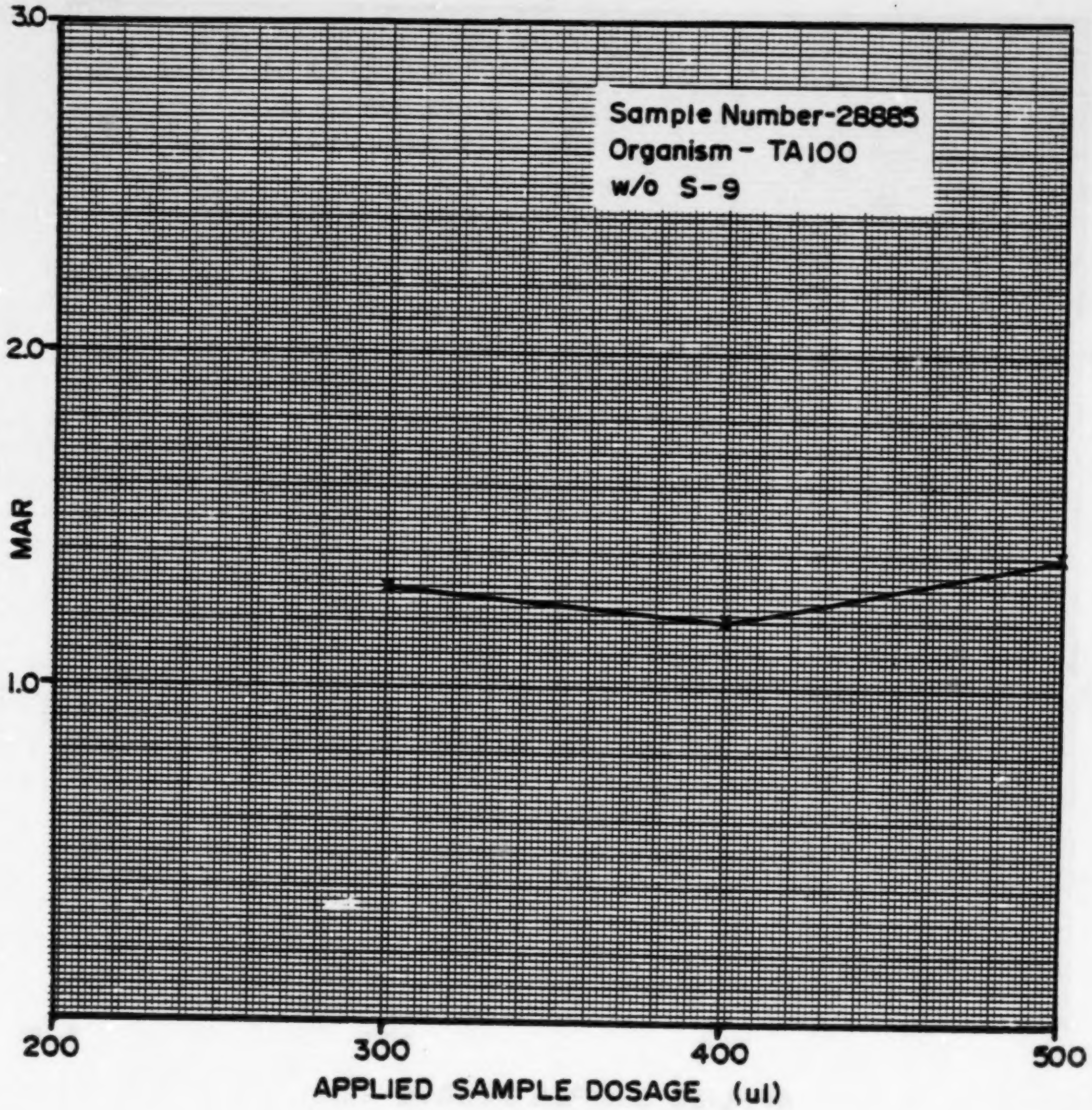

Sample Number - 94663
Organism - TA1538
w/o S-9
MAR
14.0
12.0
10.0
8.0
6.0
4.0
2.0
2X
20X
200X
EXTRACT CONCENTRATION

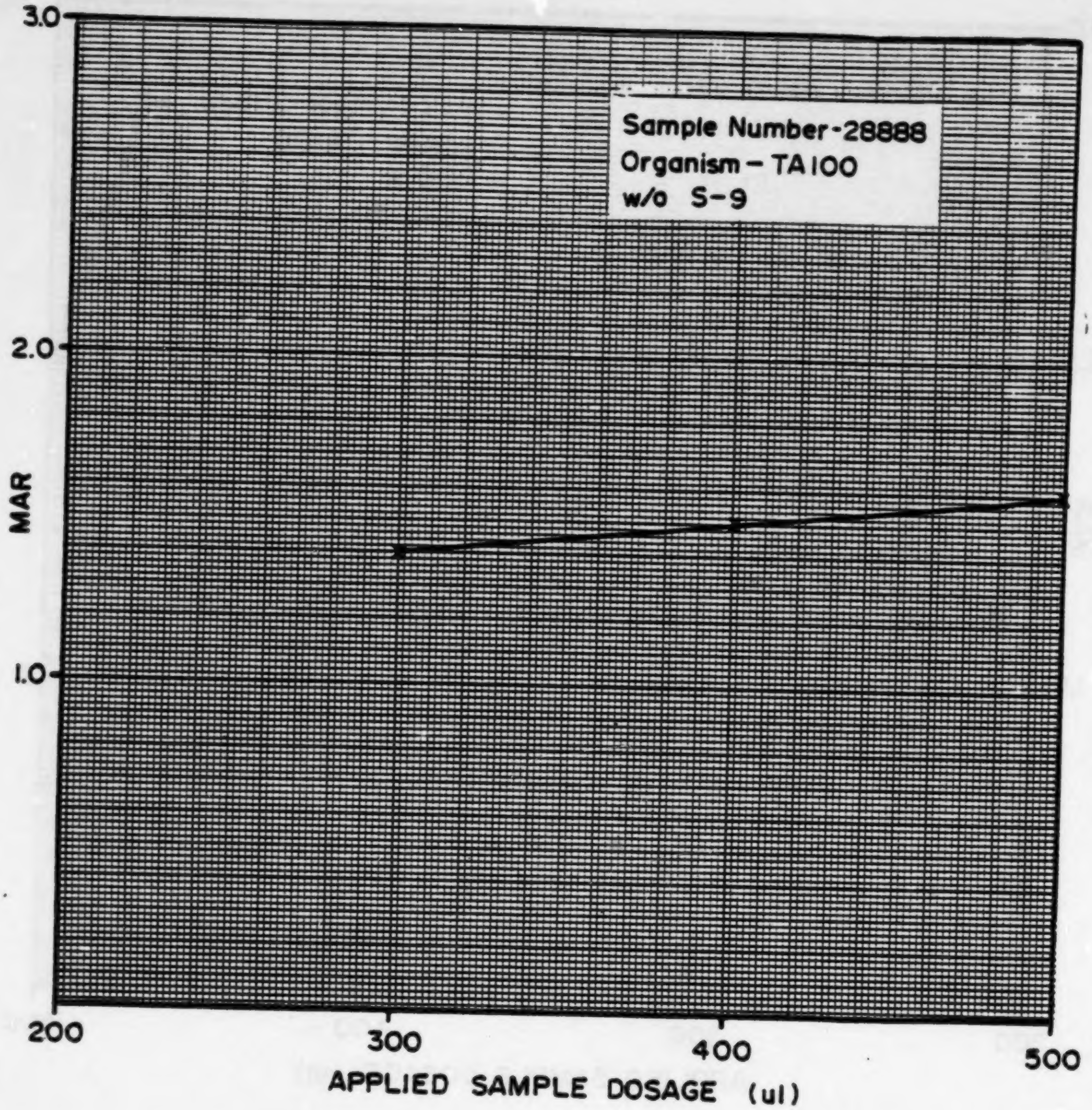
APPENDIX E

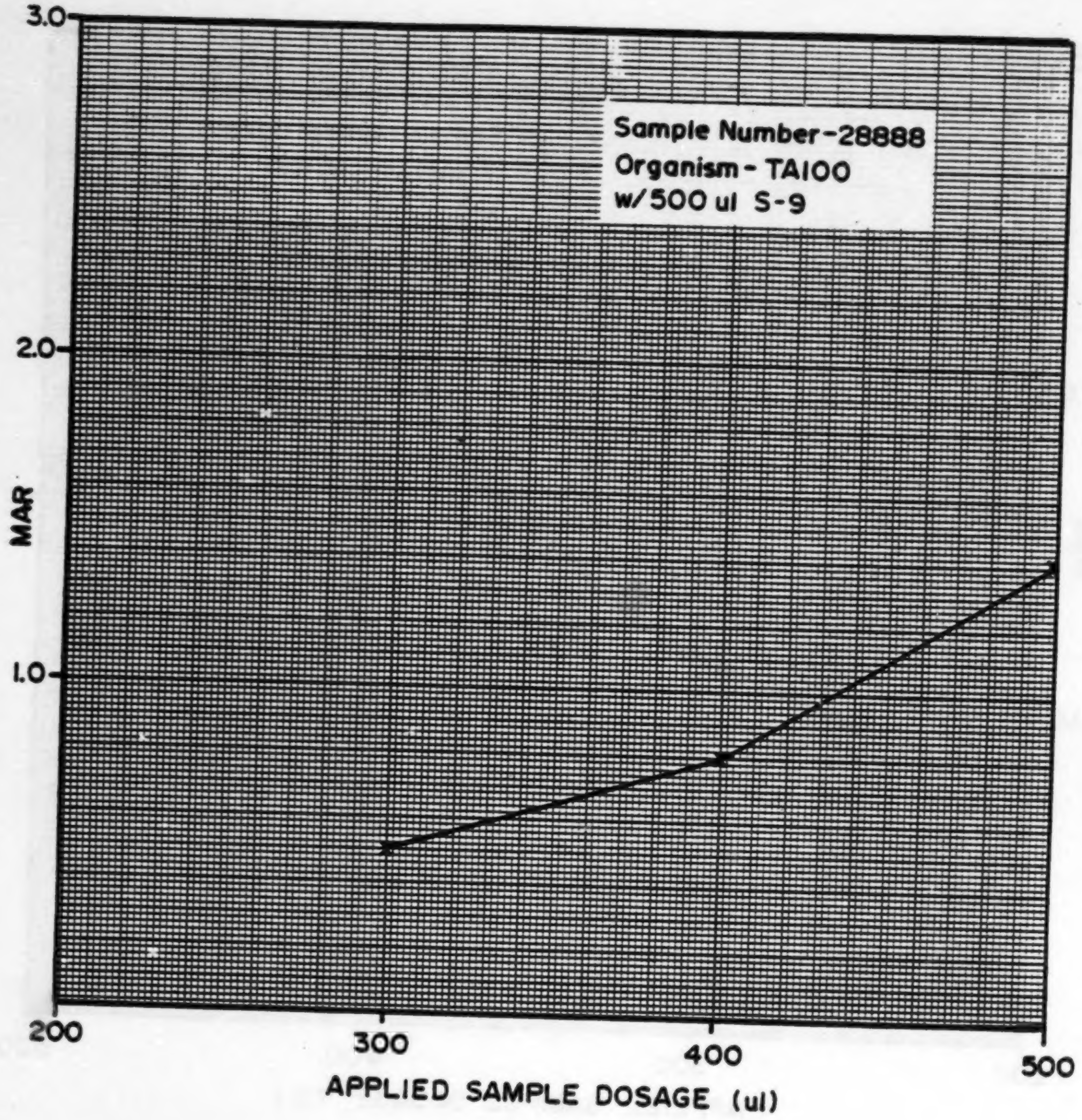
Dose Response Curves - Phase II Samples

Samples 28524 (CSO, Site 004) and 29031 (Metro influent) initially indicated a detectable response, while samples 28885 (runoff, Site 004) and 28888 (metro influent) initially exhibited toxicity to the test organisms. The samples were retested with the resultant dose responses as indicated in the following four figures.

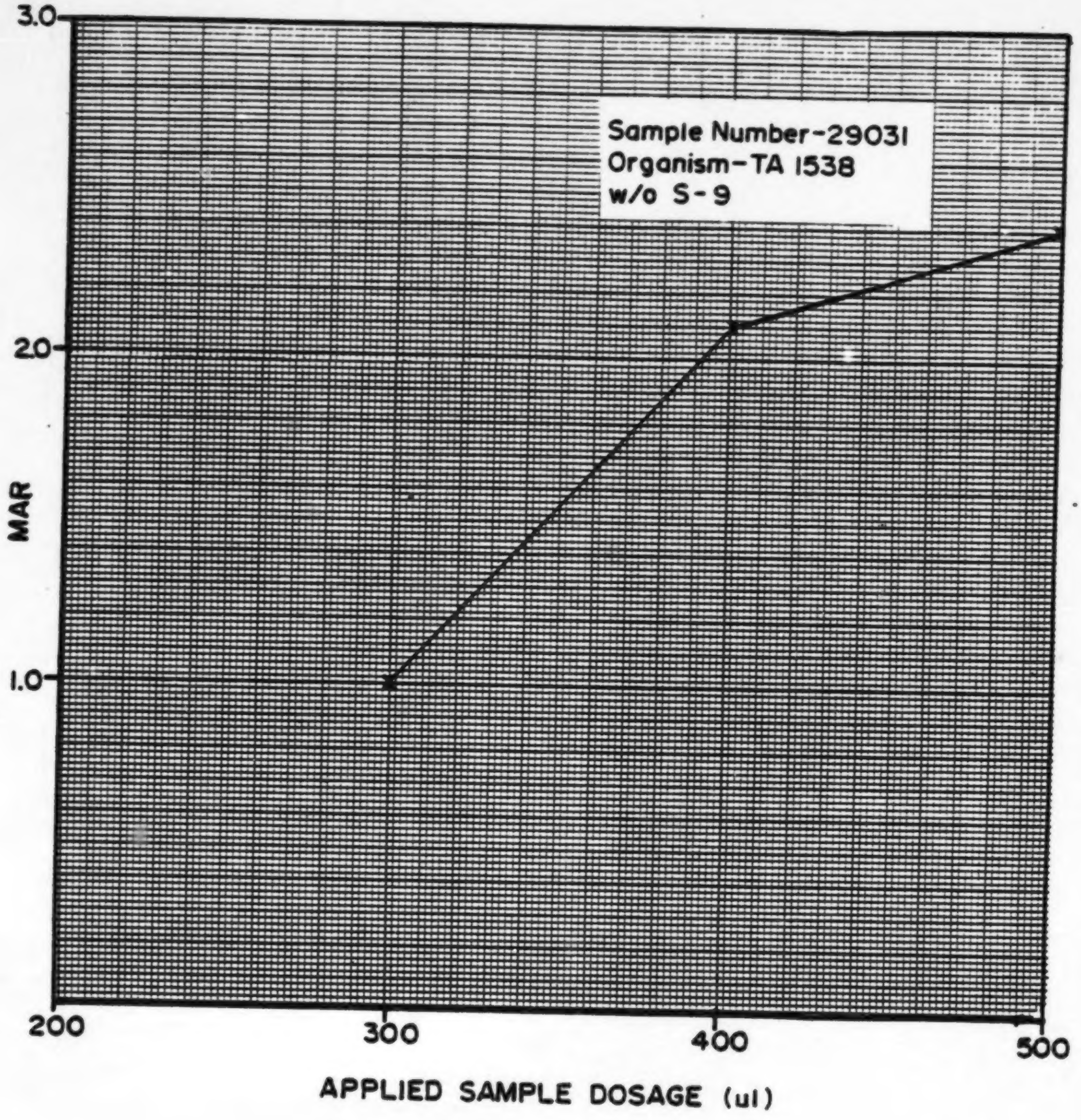

3.0
2.0
1.0
MAR
200
300
400
500
APPLIED SAMPLE DOSAGE (ul)
Sample Number-28524
Organism - TA1538
w/o S-9


Sample Number-28885
Organism - TA100
w/o S-9
3.0
2.0
1.0
MAR
200
300
400
500
APPLIED SAMPLE DOSAGE (ul)


Sample Number-28888
Organism - TA100
w/o S-9
3.0
2.0
1.0
MAR
200
300
400
500
APPLIED SAMPLE DOSAGE (ul)



Sample Number-28888
Organism - TA100
w/500 ul S-9
3.0
2.0
1.0
MAR
200
300
400
500
APPLIED SAMPLE DOSAGE (ul)


Sample Number-29031
Organism-TA 1538
w/o S-9
3.0
2.0
1.0
MAR
200
300
400
500
APPLIED SAMPLE DOSAGE (ul)